Original Article

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Microbiology Section

BioFire FilmArray Meningitis/Encephalitis Panel Utilised for the Unsolved Cases of Meningitis/Encephalitis: A Cross-sectional Study

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ABSTRACT

Introduction: Syndrome-based diagnosis of various infections is increasingly important. Even in resource-limited countries, adopting a syndrome-based method is essential, as Meningitis/Encephalitis (ME) can be caused by a multitude of agents that require detection through a multiplex assay.

Aim: To identify the causative agent in ME cases where at least one conventional microbiological method had failed.

Materials and Methods: The present study was a cross-sectional study conducted in the virology section of the Microbiology Department at the Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India. The study was carried out from June 2023 to October 2024. A total of 42 cases diagnosed by clinicians as Meningoencephalitis (ME) based on clinical findings (such as fever, headache, neck stiffness and encephalitis, which includes changes in mental status, behaviour and neurological function) were included. The Cerebrospinal fluid (CSF) from these patients had already been submitted to the microbiology lab and had returned negative results on routine CSF culture (Gram stain plus routine bacterial culture) or on routine Polymerase Chain Reaction (PCR) targeting one or two viruses (HSV/Enterovirus/VZV). These samples were subjected to the BioFire FilmArray ME Panel. For statistical analysis, continuous variables such as age and gender were interpreted as means or medians, while categorical variables were interpreted as numbers, percentages, and 95% confidence intervals. A p-value <0.05 was considered significant.

Results: Out of the 42 samples tested, organisms were detected in 14 (33.33%) samples, while 28 (66.7%) samples showed no organism. The majority of the identified organisms were viruses (9 or 21.4%), followed by bacteria (4 or 9.52%) and fungi (1 or 2.38%). There were 26 (61.9%) males and 16 (38.1%) females, with ages ranging from one month to 74 years. The most common symptoms among the patients were headache, followed by fever and nausea/vomiting.

Conclusion: A syndrome-based diagnosis of ME should be implemented in every institute, following the development of a proper diagnostic algorithm that is both feasible and accurate, based on local experience and available resources.

Keywords: Algorithm, Cerobrospinal fluid, Syndrome-based diagnosis

INTRODUCTION

The ME refers to critical conditions that require rapid and precise management. Over half of the patients who survive end up with devastating sequelae such as epilepsy, motor and sensory defects, cognitive deficits and vision and hearing impairments [1,2]. Timely diagnosis and treatment are key to avoiding these morbidities and mortality. The problem with conventional diagnostic methods is the long turnaround time, and the results depend on multiple factors, such as the timing of lumbar puncture, prior antibiotic treatment, volume of CSF analysed, temperature of storage, etc. [3-6]. Additionally, statistics show that even after exhaustive efforts for diagnosis, one-fourth to one-half of such patients remain without an aetiological diagnosis [7-10]. In such situations, a rapid multiplex PCR that targets several bacteria, viruses, and fungi is an attractive option for the diagnosis of ME.

The BioFire FilmArray ME Panel (The BioFire FilmArray® (BioFire Diagnostics, a bioMérieux Company, Salt Lake City, UT, USA) was FDA approved in October 2015 (DEN150013) for the detection of aetiological agents of meningitis and encephalitis [11]. The runtime for this assay is approximately 1 hour and it requires only 200 µL of CSF. It targets 14 pathogens: Bacteria: *Escherichia coli* K1, *Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis* (encapsulated), *Streptococcus agalactiae*, and *Streptococcus pneumoniae*. Viruses: Cytomegalovirus, Enterovirus, Herpes simplex virus 1, Herpes simplex virus 2, Human herpesvirus 6, Human parechovirus and Varicella zoster virus. Yeast: *Cryptococcus neoformans/gattii* [11]. The rationale behind designing such a study was to find a way for the judicious use of the ME panel in resource-limited settings where the BioFire FilmArray is not affordable for everyone.

So far, studies have used this assay as a first-line diagnostic test, but present study aimed to explore its use as a reserve test. This type of study can assist in devising an algorithm for future use. The primary aim of this study was to identify the causative organism of ME where the first-line conventional tests have failed to do so, with secondary objectives being to help devise an algorithm for the use of the ME panel in resource-limited settings and to evaluate whether reserving the ME panel as a second-line diagnostic test improves the positivity rate when compared with published data.

Currently, we do not have any published guidelines for the use of the ME panel. Present study was designed to evaluate the use of the ME panel in cases with a clinical diagnosis of ME based on their clinical findings (such as fever, headache, neck stiffness and encephalitis, which also involves changes in mental status, behaviour and neurological function) when the conventional culture and/or targeted PCR failed to detect any organism.

MATERIALS AND METHODS

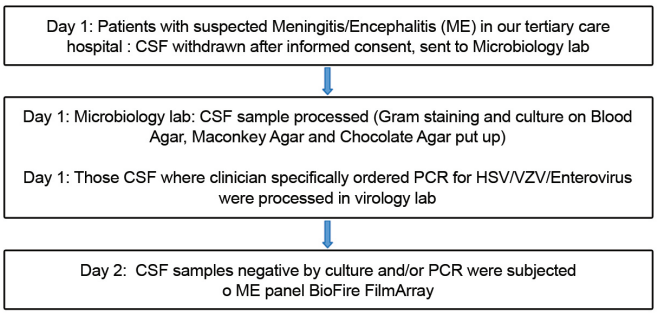
The present cross-sectional study was conducted in the virology section of the Microbiology department of the Sher-i-Kashmir

Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India. The study was carried out from June 2023 to October 2024. The study was conducted after obtaining ethical clearance from the institute's ethical committee (SIMS 131/IEC-SKIMS/2024-238). Informed consent was obtained from the patient or their relative before the lumbar puncture.

Inclusion criteria: The CSF was collected by lumbar puncture and at least one conventional microbiological test was performed on the CSF. Those CSF samples which were negative on conventional testing were included in the study.

Exclusion criteria: CSF samples taken from CSF shunts were excluded from the study.

This was a time-bound study, and total of 42 cases were recruited during the scheduled period. The workflow is depicted in [Table/Fig-1].



[Table/Fig-1]: Work flow depicted in a flow chart.

Those cases (a total of 42) that had a strong clinical suspicion of ME based on their clinical findings (such as fever, headache, neck stiffness, and encephalitis involving changes in mental status, behaviour, and neurological function) and whose CSF had already been submitted to the microbiology lab and returned negative on routine CSF culture (Gram stain plus routine bacterial culture) and/or on routine PCR targeting one or two viruses (HSV/Entero/VZV) were subjected to the BioFire FilmArray.

Processing of samples on BioFire Film Array (RFIT-ASY-0118) [12]: The BioFire FilmArray was run according to the manufacturer's guidelines. Briefly, the pouch was inserted into the loading station, then hydration solution was injected. After that, the sample, combined with the sample buffer, was injected into the pouch and finally, the pouch was inserted into the FilmArray, and the run was set up. First, the FilmArray extracts and purifies all nucleic acids from the sample. Next, the FilmArray performs a nested multiplex PCR. During the first-stage PCR, the FilmArray conducts a single, large-volume, massively multiplexed reaction. Lastly, individual singleplex second-stage PCR reactions detect the products from the first-stage PCR. Using endpoint melting curve data, the FilmArray software automatically generates a result for each target in a single report.

STATISTICAL ANALYSIS

Data were recorded in Microsoft Excel. Continuous variables such as age and gender were reported as mean or median, while categorical variables were expressed as numbers, percentages, and 95% confidence intervals.

RESULTS

A total of 42 CSF samples from patients diagnosed by clinicians with meningitis or ME based on their clinical findings (such as fever, headache, neck stiffness, and encephalitis, which also involved changes in mental status, behaviour, and neurological function) were tested. These samples had already been submitted to the microbiology lab and had returned negative on routine CSF culture (Gram stain plus routine bacterial culture) or on routine PCR targeting one or two viruses (HSV/Entero/VZV) before being subjected to the BioFire FilmArray.

There were 26 (61.9%) males and 16 (38.1%) females, with ages ranging from one month to 74 years. Demographic details are summarised in [Table/Fig-2]. The most common symptoms were headache, followed by fever and nausea/vomiting [Table/Fig-3].

Characteristics	n (%)
Gender	
Male	26 (61.9)
Female	16 (38.1)
Age (years)	
<1	5 (11.9)
1-10	10 (23.85)
11-20	10 (23.8)
21-30	3 (7.1)
31-40	3 (7.1)
41-50	2 (4.7)
51-60	3 (7.1)
61-70	5 (11.1)
>70	1 (2.38)

[Table/Fig-2]: General characteristics of patients.

Symptoms	n (%)
Fever	30 (71.4)
Headache	32 (76.2)
Abnormal body movements	10 (23.8)
Rash	12 (28.6)
Nausea/vomiting	20 (47.6)
Altered mental status/confusion	12 (28.5)
Seizure	5 (11.9)
Lethargy	4 (9.5)

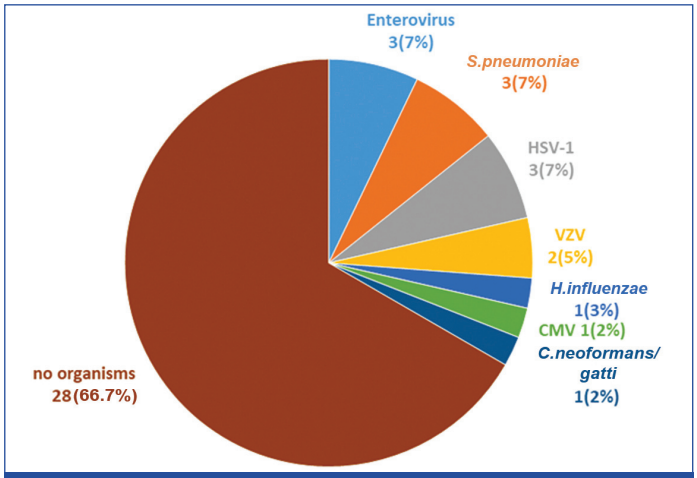
[Table/Fig-3]: Symptoms of patients presenting with meningoencephalitis.

Cerebrospinal fluid analysis showed pleocytosis (>5 WBCs/μL in CSF) in 20 (47.6%) of the patients. CSF glucose levels were normal in 35 patients, and protein levels were elevated in 30 patients; details of CSF parameters are provided in [Table/Fig-4].

Parameters	Increased n (%)	Decreased n (%)	Normal n (%)
WBC	20 (47.6)	-	22 (52.4)
Glucose	5 (11.9)	2 (4.8)	35 (83.3)
Protein	30 (71.4)	-	12 (28.6)

[Table/Fig-4]: Cerebrospinal fluid findings of the patients.

Out of the 42 samples tested, organisms were detected in 14 (33.3%) samples, whereas in 28 (66.7%) samples, no organisms were detected. The distribution of bacteria, virus and fungi is illustrated in [Table/Fig-5].



[Table/Fig-5]: Breakup of organisms detected by ME panel.

DISCUSSION

A syndrome-based approach is being practised and encouraged in standard-of-care testing in various institutes across the globe. Such an approach offers comprehensive results for most of the likely causative organisms of a particular disease, and that too in a very short span of time. Although the BioFire Film Array ME Panel was introduced with the aim of quick and rapid diagnosis of ME [11], present study sought to explore another aspect of the panel, which is to reserve it as a second-line test in strongly suspected ME patients where one of the conventional microbiological test results was negative.

In various studies conducted in India and other countries, the positivity rate ranges from 7 to 23% [Table/Fig-6] [13-18]. Present study had a significantly higher positivity rate (33.3%) compared to others, which may be attributed to the very stringent patient selection criteria in our research. There were still many cases that remained undiagnosed; the reasons for this may be attributed to factors such as immune neutralisation, post-infectious mechanisms and the limited scope of the assay, among others.

Author	Year of publication	Total samples tested	Total positives (%)	Place of study
Present study	2025	42	14 (33.3)	SKIMS
Ota K et al., [13]	2023	70	18 (26)	China
Chandran S et al., [14]	2022	259	61 (23.6)	India
Lagamayo EN and Rusia-Uy RO [15]	2021	143	20 (14)	Phillipines
Boudet A et al., [16]	2019	734	89 (12.1)	France
Lee SH et al., [17]	2019	42	6 (7)	China
Radmard S et al., [18]	2019	705	45 (6.38)	USA

[Table/Fig-6]: Positivity rate of BioFire FilmArray Meningitis/Encephalitis (ME) Panel in various studies across the globe [13-18].

Other causes for a negative test may include organisms not included in the panel, such as *Mycobacterium tuberculosis*, a low bioburden, and antibiotic treatment administered prior to testing. In present study, the majority of organisms detected were viruses (9), which explains the reason for having a negative conventional culture. In four of these cases, antivirals were initiated and antibiotics were discontinued; however, in the remaining five cases, clinicians chose to continue antibiotic treatment.

In a study conducted by Radmard S et al., in New York over a period of one year, 30 out of 45 positive cases were of viral aetiology [18]. The detection of viruses is also very significant because some viral infections, like herpes simplex virus infection, if not treated promptly with acyclovir, can lead to cerebral invasion [19].

Present study also recovered bacteria in four cases, three of which were *Streptococcus pneumoniae* and one *Haemophilus influenzae*. In all these cases, the antibiotics were modified according to the organism and our hospital antibiogram. The reason for their not growing on conventional culture can be attributed to their fastidious growth requirements or the patient already being on antibiotics. Such infections also have a high potential to be fatal if not promptly treated with antibiotics [19].

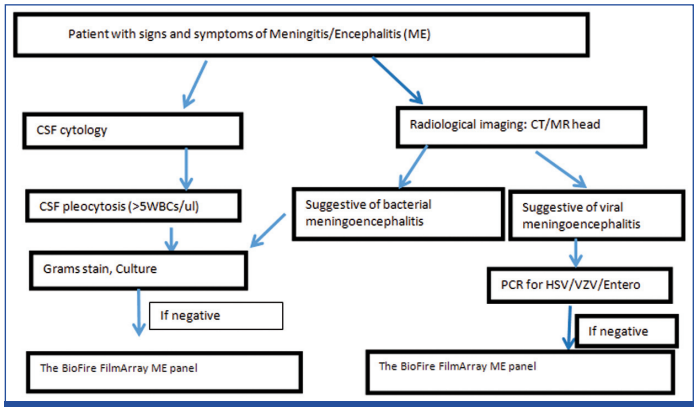
Additionally, present study encountered a case of cryptococcal meningitis in an 18-year-old asthmatic boy who initially presented with lymphadenitis and later developed signs of meningitis. The ME panel yielded a result of *Cryptococcus neoformans/gattii.* His CSF sample was processed in the bacT/ALERT system, which grew yeast after three days of incubation and was identified by VITEK II as *Cryptococcus neoformans*. The patient was started on injectable amphotericin B and flucytosine, to which he responded well. A repeat culture was conducted after four weeks, which turned out to be negative, and he was subsequently placed on maintenance therapy. These findings emphasise the importance of using the ME panel in difficult-to-diagnose cases.

In present study, CSF pleocytosis (total WBCs >5/µL) was detected in 47.6% of cases, while 52.4% did not show CSF pleocytosis. There are studies that propose including CSF pleocytosis as a criterion for case selection in the patient care algorithm [19]. Whenever a diagnostic test is used, there should be clear guidelines for test indications. In places where cost is not a significant factor, this panel has been reported to be overused, leading to a wastage of resources and confusion regarding results [20,21]. Therefore, judicious use of such a test is essential, and this panel should be interpreted while considering the clinical picture of the patient, as well as the laboratory and neuroimaging findings.

This particular panel has also been explored for other benefits, such as reduction in days of acyclovir therapy, discontinuation of vancomycin as part of antimicrobial stewardship, and overall cost efficiency, among others [22].

However, there is a problem of false positives and false negatives with this panel, as reported by other studies. A study on the differential performance of ME panels by Schnuriger A et al., raised concerns about the suboptimal performance of FilmArray for the detection of enterovirus and HSV-1 [19].

Based on our limited experience, authors propose a diagnostic algorithm to be used in resource-limited settings, depicted in [Table/Fig-7].



[Table/Fig-7]: Proposed diagnostic algorithm for use of BioFire FilmArray Meningitis/Encephalitis (ME) Panel in resource limited set-up.

Limitation(s)

The limitations of this study included a small sample size. Another limitation was that this panel was not used as a first-line diagnostic test. There are also limitations to this panel, such as a restricted range of pathogens, particularly in Indian settings where there is a high burden of *Mycobacterium tuberculosis*. Additionally, only one fungus is included in the panel, omitting a long list of fungal pathogens that can cause meningitis, such as *Candida, Histoplasma*, and others.

CONCLUSION(S)

The BioFire FilmArray ME Panel is a significant advancement in the diagnosis of ME due to its rapid turnaround time and the small volume of CSF required. Based on our experience, authors propose that an institutional algorithm for the diagnosis of ME should be developed with input from all stakeholders to ensure the optimal use of such a panel. It should be utilised as an adjunctive diagnostic tool and not as a stand-alone test to guide medical decisions. Authors also recommend modifying the target organisms based on the population and the specific region being addressed.

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REFERENCES

[1] Edmond K, Clark A, Korczak VS, Sanderson C, Griffiths UK, Rudan I. Global and regional risk of disabling sequelae from bacterial meningitis: A systematic review and meta-analysis. Lancet Infect Dis. 2010;10(5):317-28. Doi: 10.1016/S1473-3099(10)70048-7. PMID: 20417414.

[2] Misra UK, Kalita J, Bhoi SK. Spectrum and outcome predictors of central nervous system infections in a neurological critical care unit in India: A retrospective review. Trans R Soc Trop Med Hyg. 2014;108(3):141-46. Doi: 10.1093/trstmh/tru008. PMID: 24535151.

[3] Cizman M, Jazbec J. Etiology of acute encephalitis in childhood in Slovenia. Pediatr Infect Dis J. 1993;12(11):903-08. Doi: 10.1097/00006454-199311000-00002. PMID: 8265278.

[4] Sivertsen B, Christensen PB. Acute encephalitis. Acta Neurol Scand. 1996;93(2-3):156-59. Doi: 10.1111/j.1600-0404.1996.tb00192.x. PMID: 8741136.

[5] Khetsuriani N, Holman RC, Anderson LJ. Burden of encephalitis-associated hospitalizations in the United States, 1988-1997. Clin Infect Dis. 2002;35(2):175-82. Doi: 10.1086/341301. Epub 2002 Jun 21. PMID: 12087524.

[6] Shukla B, Aguilera EA, Salazar L, Wootton SH, Kaewpoowat Q, Hasbun R. Aseptic meningitis in adults and children: Diagnostic and management challenges. J Clin Virol. 2017;94:110-14. Doi: 10.1016/j.jcv.2017.07.016.

[7] George BP, Schneider EB, Venkatesan A. Encephalitis hospitalization rates and inpatient mortality in the United States, 2000-2010. PLoS One. 2014;9(9):e104169. Doi: 10.1371/journal.pone.0104169. PMID: 25192177; PMCID: PMC4156306.

[8] Hasbun R, Rosenthal N, Balada-Llasat JM, Chung J, Duff S, Bozzette S, et al. Epidemiology of meningitis and encephalitis in the United States, 2011-2014. Clin Infect Dis. 2017;65(3):359-63. Doi: 10.1093/cid/cix319. PMID: 28419350.

[9] Hasbun R, Wootton SH, Rosenthal N, Balada-Llasat JM, Chung J, Duff S, et al. Epidemiology of Meningitis and Encephalitis in Infants and Children in the United States, 2011-2014. Pediatr Infect Dis J. 2019;38(1):37-41. Doi: 10.1097/INF.0000000000002081. PMID: 30531527.

[10] Bloch KC, Glaser CA. Encephalitis surveillance through the emerging infections program, 1997-2010. Emerg Infect Dis. 2015;21(9):1562-67. Doi: 10.3201/eid2109.150295. PMID: 26295485; PMCID: PMC4550161.

[11] Marketing of the first nucleic acid-based test to detect multiple pathogens from a single sample of cerebrospinal fluid. (2015) https://www.fda.gov/.

[12] The BioFire FilmArray Meningitis/Encephalitis (ME) Panel. https://www.biofiredx.com/products/the-filmarray-panels/filmarrayme/. Accessed 5/26/2025.

[13] Ota K, Fujiwara S, Ishii J, Yoshimura H, Kohara N, Kawamoto M. Efficacy of FilmArray® ME panel for the rapid diagnosis of meningitis and encephalitis. Rinsho Shinkeigaku. 2023;63(8):528-31. Japanese. Doi: 10.5692/clinicalneurol.cn-001840. Epub 2023 Jul 29. PMID: 37518019. 2023;63(8):528-31.

[14] Chandran S, Arjun R, Sasidharan A, Niyas VK, Chandran S. Clinical performance of filmarray meningitis/encephalitis multiplex polymerase chain reaction panel in central nervous system infections. Indian J Crit Care Med. 2022;26(1):67-70. Doi: 10.5005/jp-journals-10071-24078. PMID: 35110847; PMCID: PMC8783244.

[15] Lagamayo EN, Rusia-Uy RO. A two-year review of the use of film array in a tertiary hospital in the Philippines. Arch of Pulmonol Respir Care. 2021;001-06.

[16] Boudet A, Pantel A, Carles MJ, Boclé H, Charachon S, Enault C, et al. A review of a 13-month period of FilmArray Meningitis/Encephalitis panel implementation as a first-line diagnosis tool at a university hospital. PLoS One. 2019;14(10):e0223887. Doi: 10.1371/journal.pone.0223887. PMID: 31647847; PMCID: PMC6812749.

[17] Lee SH, Chen SY, Chien JY, Lee TF, Chen JM, Hsueh PR. Usefulness of the FilmArray meningitis/encephalitis (M/E) panel for the diagnosis of infectious meningitis and encephalitis in Taiwan. J Microbiol Immunol Infect. 2019;52(5):760-68. Doi: 10.1016/j.jmii.2019.04.005. Epub 2019 Apr 30. PMID: 31085115.

[18] Radmard S, Reid S, Ciryam P, Boubour A, Ho N, Zucker J, et al. Clinical utilization of the filmarray Meningitis/Encephalitis (ME) multiplex Polymerase Chain Reaction (PCR) assay. Front Neurol. 2019;10:281. Doi: 10.3389/fneur.2019.00281. PMID: 30972012; PMCID: PMC6443843.

[19] Schnuriger A, Vimont S, Godmer A. Differential performance of the filmarray meningitis/encephalitis assay to detect bacterial and viral pathogens in both pediatric and adult populations. Microbiol Spectr. 2022;10(2):e0277421. Doi:10.1128/spectrum.02774-21.

[20] Broadhurst MJ, Dujari S, Budvytiene I, Pinsky BA, Gold CA, Banaei N. Utilization, yield, and accuracy of the filmarray meningitis/encephalitis panel with diagnostic stewardship and testing algorithm. J Clin Microbiol. 2020;58(9):e00311-20. Doi: 10.1128/JCM.00311-20. PMID: 32493787; PMCID: PMC7448656.

[21] Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, et al. Multicenter evaluation of biofire filmarray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. J Clin Microbiol. 2016;54(9):2251-61. Doi: 10.1128/JCM.00730-16. Epub 2016 Jun 22. PMID: 27335149; PMCID: PMC5005480.

[22] Hagen A, Eichinger A, Meyer-Buehn M, Schober T, Huebner J. Comparison of antibiotic and acyclovir usage before and after the implementation of an on-site FilmArray meningitis/encephalitis panel in an academic tertiary pediatric hospital: A retrospective observational study. BMC Pediatr. 2020;20(1):56. Doi: 10.1186/s12887-020-1944-2. PMID: 32020860; PMCID: PMC7001287.

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