

# Optimization of the Diagnosis of Central Nervous System Infections in Vietnamese Hospitals: Results From a Retrospective Multicenter Study

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**Introduction.** Central nervous system infections pose significant health challenges, particularly in low- and middle-income countries, because of high morbidity and mortality rates. Rapid and accurate diagnosis is essential for effective treatment to prevent adverse outcomes. Traditional culture-based diagnostics are often slow and lack specificity. This study evaluates the BioFire FilmArray Meningitis/Encephalitis (FAME) Panel against standard diagnostics in Vietnam to assess its clinical impact and suitability for local epidemiology.

**Methods.** We conducted a prospective study involving 330 patients with suspected central nervous system infections at 4 hospitals in northern Vietnam from July 2022 to April 2023. Cerebrospinal fluid samples were analyzed using routine culture methods and FAME. We compared pathogen detection rates and assessed the potential clinical impact of FAME results on patient management.

**Results.** Of the 330 cerebrospinal fluid specimens, 64 (19%) were positive by either conventional diagnostics (n = 48) and/or FAME (n = 33). The agreement between FAME and conventional diagnostics was 87%. Key pathogens *Mycobacterium tuberculosis* (n = 7), *Klebsiella pneumoniae* (n = 5), *Streptococcus suis* (n = 5), Epstein-Barr virus (n = 3), *Acinetobacter baumannii* (n = 1), and *Trichosporon asahii* (n = 1) were not detected by FAME. Classical meningitis parameter clinical symptoms, altered glucose, protein, and pleocytosis were good predictors of FAME positivity, indicating their utility in optimizing local diagnostic algorithms.

**Conclusions.** FAME complements traditional diagnostics by offering rapid and broad pathogen detection, crucial for timely and appropriate therapy. However, its effectiveness varies with local epidemiology, and it should not replace conventional methods entirely. Tailoring diagnostic panels to regional pathogen prevalence is recommended to enhance diagnostic accuracy and clinical outcomes in low- and middle-income countries.

**Keywords.** cerebrospinal fluid (CSF); CNS infections; diagnostics; FAME; Vietnam.

Central nervous system (CNS) infections represent 1 of the more severe infections associated with high morbidity and

mortality, particularly in low- and middle-income countries (LMICs) [1]. Timely diagnosis and appropriate antibiotic therapy are crucial for effectively managing CNS infections because any delay in initiating such treatment is associated with unfavorable outcomes and potential sequelae [2]. Surviving individuals may experience substantial long-term consequences such as cognitive deficit, bilateral hearing loss, motor deficit, seizures, and visual impairment [3].

The diagnosis of CNS infections is challenging and typically involves the collection of cerebrospinal fluid (CSF) by lumbar puncture for culture-based microbiological diagnostics. Routine CSF diagnostics, including evaluation of cytological and biochemical parameters and clinical features, can provide insight into the etiology of the infection and attempt to distinguish between bacterial, viral, or fungal origins [4]. However, these parameters often lack the specificity to distinguish the

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infecting agent for informed decisions on appropriate empirical antimicrobial therapy. Consequently, this limitation often results in the widespread prescription of broad-spectrum antibiotics, contributing to selection pressure and the emergence of antibiotic resistance.

In addition, the traditional culture-based microbiological approach, suffers from time constraints and potential sensitivity issues, especially when patients have received prior antibiotic treatment [5]. Recognizing these limitations, molecular diagnostics have emerged as a valuable addition to the diagnostic toolkit because of their rapid turnaround time. Most commercially available molecular tests for the diagnosis of CNS infections are targeted polymerase chain reaction (PCR) assays designed to identify the most common pathogens. However, it is important to note that these tests often reflect general epidemiologic trends rather than being tailored to specific regional contexts, particularly in LMICs where epidemiologic patterns can differ significantly from those in high-income countries. In particular, there has been limited research in resource-limited settings, with only 1 study identified in Myanmar [6], whereas other studies in Asia have predominantly been conducted in non-LMIC settings, such as Taiwan [7] and Korea [8].

In this context, we evaluated the additional benefit of implementing the BioFire FilmArray Meningitis/Encephalitis Panel (FAME) compared with the standard-of-care diagnostics (culture and an in-house multiplex PCR panel) in 4 hospitals around Hanoi, northern Vietnam. The primary objective was to investigate whether the targets included in FAME were suitable for the local epidemiology in Vietnam; the secondary objective was to assess whether the implementation of such a panel would have had a meaningful clinical impact.

## METHODS

### Ethical Approval Statement

Written informed consent was obtained from all hospitalized patients and/or their relatives and from parents if subjects were age <18 years old. The study protocol was approved by the 108 Military Central Hospital (108 MCH) institutional review board (108MCH/RES/MENTNGITIS-V-D3-25042017). All experiments were conducted in accordance with International Council for Harmonization-Good Clinical Practice/Good Clinical Laboratory Practice (ICH-GCP/GCLP) guidelines and regulations.

**Study Population and Study Design.** Patients with suspected CNS infections with clinical signs of CNS infection were recruited prospectively in 4 hospitals in northern Vietnam, including 108 MCH, National Hospital for Tropical Diseases (NHTD), 103 Military Hospital (103 MH), Hanoi, and Viet Tiep Friendship Hospital (VT), Haiphong, Vietnam, between 1 July 2022 and 30 April 2023. Patients were eligible for the study if

they had clinical signs of suspected CNS infections, based on the World Health Organization case definition modified by Dubot-Pères et al [9]. Patient recruitment was at the discretion of the treating physician and followed local clinical practice. The inclusion criteria required patients to have a fever or an axillary temperature >37.5 °C and to exhibit a combination of at least 2 of the following symptoms: focal neurologic deficits, Glasgow Coma Scale abnormalities, seizures, neck stiffness, and signs of altered mental status. Exclusion criteria included: (1) patients with noninfectious, noninflammatory neurological disorders; patients not presenting to the emergency department; patients transferred from other hospitals; or those lacking an acute indication for a lumbar puncture in the emergency department; patients with contraindications to lumbar puncture, such as an intracranial space-occupying lesion with mass effect, a mass in the posterior fossa, abnormal intracranial pressure, or a local skin infection at the lumbar puncture site; (2) patients with an incomplete clinical history; and (3) patients who have not consented to the study.

A total of 330 CSF samples from 330 patients with suspected CNS infections were analyzed (199 samples collected on admission and 131 during hospitalization). Case record forms were used to collect data on demographics, medical history, clinical features on admission and subclinical findings on admission or during hospitalization, clinical course, treatment, outcome, and neurological findings on discharge. Patients were diagnosed and treated according to the clinical algorithm and management of the respective hospital. Clinical outcome was defined according to the Glasgow Outcome Scale [10]. The CSF samples were collected at various hospital sites in Vietnam and stored at –80 °C. They were then transported to Germany, where all CSF samples were analyzed using the FAME assay. The cold chain was rigorously maintained throughout the entire process to ensure the samples integrity during transportation.

**Standard-of-care Laboratory Diagnostics.** Routine CSF tests included cell counts and differential counts, glucose and total protein analyses and CSF bacterial cultures. If required, additional tests such as CSF fungal culture and polymerase chain reaction (PCR) tests for specific bacteria, and viruses were also performed. The microbiological culture procedures were similar on all study sites. Briefly, 1 mL of CSF was collected from a lumbar puncture for microbiological culture. Cultures were performed using the BACTEC Plus Aerobic/F System (Becton–Dickinson, Franklin Lakes, NJ, USA) at 36 °C with CO<sub>2</sub> for 18–72 hours. Each positive culture was grown on a selective medium such as blood agar, chocolate agar, and MacConkey agar (Merck, Kenilworth, NJ, USA). When bacterial growth was detected, colonies were selected for species identification using the matrix-assisted laser desorption/ionization time of flight VITEK MS system for automated microbial identification and antimicrobial susceptibility testing was performed using the automated VITEK<sup>®</sup>2 compact system

(BioMérieux, Lyon, France). PCR is performed at the request of physicians, in addition to routine diagnostics. An overview of the coverage of the PCR panel at the different study sites is summarized in [Supplementary Table 1](#).

**BioFire FilmArray Meningitis/Encephalitis Assays.** All frozen CSF samples collected from patients by lumbar puncture were analyzed with FAME following manufacturer's instructions. In brief, frozen, noncentrifuged CSF (200  $\mu$ L) was placed in the bag after the hydration solution had been injected and processed according to the manufacturer's instructions. FAME panel included 14 pathogens, namely *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Escherichia coli* K1, herpes simplex virus (HSV) 1 and HSV 2, human herpes virus 6, cytomegalovirus (CMV), Enterovirus, human parechovirus, varicella zoster virus (VZV), and *Cryptococcus neoformans/gattii*.

**Data Analysis.** Descriptive analysis was performed using SPSS Statistics 28 (IBM). The presence of pleocytosis, was quantitatively defined by 2 separate cutoff ranges for the corrected white blood cell count in CSF:  $\geq 5$  cells/ $\text{mm}^3$  and  $\geq 10$  cells/ $\text{mm}^3$  [11]. Abnormal CSF glucose and protein levels were defined for all patients as values of  $<2.8$  mmol/L or  $>4.2$  mmol/L and  $<0.10$  g/L and  $>0.25$  g/L, respectively, based on the 108 MCH criteria. Discordant results were defined as divergent results only when the pathogen was included in the standard of care diagnostic panel. The association of the clinical parameters with the positivity of detection by conventional or FAME method was evaluated using a Random Forest model using the positive/negative status as a prediction of the importance (Gini) of all available clinical parameters as predictors using the package Random Forest in R 4.3.3. Missing values were handled using the command `na.roughfix`, which replaced the quantitative missing value with the overall population medians and the qualitative values with the most frequent values in the population.

## RESULTS

### General Characteristics of the Study Cohort

A total of 330 patients from 456 patients with suspected CNS infections (111 fulfilling exclusion criteria and 17 nonconsent) were recruited in various hospitals around Hanoi, Vietnam ([Supplementary Figure 1](#)). Recruitment numbers varied by site: 40 patients from 103 MH, 52 from 108 MH, 136 from the NHTD, and 102 from VT Hospital. At 108 MH, 52 of 81 eligible patients were recruited, whereas the remaining 29 were excluded because of reasons such as transfer from other hospitals ( $n = 6$  patients), incomplete clinical histories ( $n = 6$ ), death ( $n = 2$ ), not presenting directly to the emergency department ( $n = 4$ ), lack of consent ( $n = 5$ ), and family decisions to

take elderly patients home ( $n = 6$ ). At NHTD, 136 of 175 patients were recruited, with 6 declining consents and 33 fulfilling the exclusion criteria. At 103 MH, 40 of 60 patients were recruited, with 2 not consenting and 18 fulfilling the exclusion criteria. No further data were available from these 2 hospitals. At VT, 102 of 140 patients were recruited, with the remaining 38 patients excluded because of contraindications to lumbar puncture ( $n = 5$ ), transfers from other hospitals ( $n = 13$ ), lack of consent ( $n = 4$ ), diagnosis was uncertain and inconclusive ( $n = 7$ ), and no retrievable clinical history ( $n = 9$ ). The median age of the cohort was 54 years (range, 11–97 years). Of these, 225 (68%) were male. The main clinical characteristics are summarized in [Table 1](#). Regarding preexisting medical conditions, 27% of patients had hypertension, 19% had diabetes, and 6% had cardiac disease. Common clinical presentations included fever (83%), headache (67%), neck stiffness (63%), and altered mental status (defined by a Glasgow Coma Scale score below 14) in 43% of cases. Notably, 83% of patients had at least 2 of the following symptoms: headache, fever, neck stiffness, and altered mental status.

### Detected Pathogens by FAME and Conventional Diagnostics

In total, 64 of 330 (19%) samples yielded positive results for bacterial, fungal, and/or viral pathogens. In our study, tuberculous meningitis was the leading cause of community-acquired CNS infections with community onset in 7 of 8 positive cases. The leading causes of community-acquired bacterial CNS infections were *S pneumoniae* with community-onset in 5 of 6, *Streptococcus suis* with 4 of 5, and *H influenzae* with 4 of 5 cases. Meanwhile, *Klebsiella pneumoniae* with hospital-onset in 5 of 7 positive cases, is the most common bacterial pathogen detected in hospital-acquired CNS infections. Among viral pathogens, VZV and HSV were predominant and equally represented in community-onset and hospital-onset viral CNS infections. *C neoformans* was the most common fungal pathogen but was only sporadically detected. *E coli*, HSV-2, human herpes virus 6 and human parechovirus were not detected in the study with FAME ([Figure 1](#)).

The laboratory procedures for culture-based diagnostics were consistent across all 4 study sites. However, there were variations in the range of pathogens covered by the local molecular diagnostics (PCR) panels ([Supplementary Table 1](#)). Consequently, the evaluation of the results of the FAME and the conventional diagnostics results (culture plus molecular method) differed between the centers ([Figure 2](#)). Overall, in 48 CSF samples, pathogens were detected by conventional method and in 33 samples, a positive result was obtained in the FAME.

The positivity rates at the different study sites were 10% (4/40) for 103 MH, 37% (19/52) for 108 MCH, 24% (33/136) for NHTD, and 8% (8/102) for VT, detected by either conventional methods or FAME. As expected, the highest concordance was observed at 108 MCH, which had the most extensive PCR panel, with 63% (5/8 pathogens) concordance in

**Table 1. Demographic and Clinical Characteristics of Vietnamese Patients With Central Nervous System Infections**

General Characteristics	CNS Infections (n = 330)	Routine Positive Specimens (n = 48)	Routine Negative Specimens (n = 282)	P Value	FAME-positive Specimens (n = 33)	FAME-negative Specimens (n = 297)	P Value
<b>Demographics</b>							
Age (mean ± SD)	54 ± 19	53 ± 18	55 ± 19	.47	53 ± 18	55 ± 19	.62
Male sex—no. (%)	225 (68)	37 (77)	188 (67)	.15	24 (73)	201 (68)	.56
Underlying condition—no. (%)							
Hypertension	90 (27)	8 (17)	82 (29)	.07	10 (30)	80 (27)	.68
Diabetes	62 (19)	2 (4)	60 (21)	.005	4 (12)	58 (20)	.30
Cardiac disease	19 (6)	1 (2)	18 (6)	.33 <sup>a</sup>	0 (0)	19 (6)	.24 <sup>a</sup>
Alcoholism	17 (5)	8 (17)	9 (3)	<.001 <sup>a</sup>	2 (6)	15 (5)	.68 <sup>a</sup>
Chronic liver disease	18 (6)	4 (8)	14 (5)	.31 <sup>a</sup>	1 (3)	17 (6)	1 <sup>a</sup>
Chronic lung disease	17 (5)	3 (6)	14 (5)	.72 <sup>a</sup>	3 (9)	14 (5)	.24 <sup>a</sup>
Kidney disease	15 (5)	2 (4)	13 (5)	1 <sup>a</sup>	1 (3)	14 (5)	1 <sup>a</sup>
Immunosuppressive drugs	13 (4)	1 (2)	12 (4)	.7 <sup>a</sup>	1 (3)	12 (4)	1 <sup>a</sup>
Cancer	13 (4)	0 (0)	13 (5)	.23 <sup>a</sup>	0 (0)	13 (4)	.38 <sup>a</sup>
HIV-positive	9 (3)	1 (2)	8 (3)	1 <sup>a</sup>	0 (0)	9 (3)	.61 <sup>a</sup>
Risk factor—no. (%)							
Post neurosurgery	29 (9)	5 (10)	24 (9)	.59 <sup>a</sup>	3 (9)	26 (9)	1 <sup>a</sup>
Head trauma	31 (9)	2 (4)	29 (10)	.28 <sup>a</sup>	3 (9)	28 (9)	1 <sup>a</sup>
CSF shunt	5 (2)	0 (0)	5 (2)	1 <sup>a</sup>	0 (0)	5 (2)	1 <sup>a</sup>
Clinical features—no. (%)							
Fever (>37.5 °C)	273 (83)	46 (96)	227 (81)	.009	32 (97)	241 (81)	.02
Headache	221 (67)	37 (77)	184 (65)	.11	27 (82)	194 (65)	.06
Neck stiffness	207 (63)	40 (83)	167 (59)	.001	27 (82)	180 (61)	.02
Nausea/vomiting	99 (30)	21 (44)	78 (8)	.025	13 (39)	86 (29)	.21
Seizure	29 (9)	3 (6)	26 (9)	.78 <sup>a</sup>	1 (3)	28 (9)	.34 <sup>a</sup>
Focal neurologic deficits	20 (6)	4 (8)	16 (6)	.51 <sup>a</sup>	2 (6)	18 (6)	1 <sup>a</sup>
Glasgow Coma Score	13 ± 2	12 ± 2	13 ± 2	<.001	13 ± 2	13 ± 2	.62
< 14 (indicating altered mental status)—no. (%)	142 (43)	36 (75)	106 (38)	<.001	19 (58)	123 (41)	.08
At least 2 of 4 symptoms (headache, fever, stiff neck, and altered mental status)	273 (83)	45 (94)	228 (81)	.03	32 (97)	241 (81)	.02
<b>Outcome</b>							
Death	32 (10)	4 (8)	28 (10)		3 (9)	29 (10)	
Vegetative state	29 (9)	10 (21)	19 (7)		3 (9)	26 (9)	
Severe disability	64 (19)	8 (17)	56 (20)		4 (12)	60 (20)	
Moderate disability	62 (19)	5 (10)	57 (20)		5 (15)	57 (19)	

**Table 1. Continued**

General Characteristics	CNS Infections (n = 330)	Routine Positive Specimens (n = 48)	Routine Negative Specimens (n = 282)	P Value	FAME-positive Specimens (n = 33)	FAME-negative Specimens (n = 297)	P Value
Mild or no disability	143 (43)	21 (44)	122 (43)		18 (55)	125 (42)	
Cerebrospinal fluid							
Cell count (cells/mm <sup>3</sup> ) <sup>b</sup>							
mean ± SD	1032 ± 5897	4898 ± 14437	376 ± 1726	<.001	3315 ± 7751	761 ± 5601	.007
Pleocytosis of 5 cells/mm <sup>3</sup>	225 (69)	42 (89)	183 (66)	.001	29 (88)	196 (67)	.015
Pleocytosis of 10 cells/mm <sup>3</sup>	175 (54)	39 (83)	136 (49)	<.001	26 (79)	149 (51)	.003
Protein (g/L) <sup>c</sup>							
Mean ± SD	1.56 ± 2.78	3.54 ± 4.01	1.22 ± 2.36	<.001	2.38 ± 2.36	1.47 ± 2.82	.07
Abnormal protein	307 (94)	48 (100)	259 (93)	.089 <sup>a</sup>	33 (100)	274 (94)	.24 <sup>a</sup>
Glucose (mmol/L) <sup>c</sup>							
Mean ± SD	4.16 ± 2.96	2.11 ± 1.85	4.50 ± 2.98	<.001	3.35 ± 3.85	4.25 ± 2.84	.09
Abnormal glucose	188 (58)	36 (77)	152 (54)	.005	23 (70)	165 (56)	.10

Abbreviation: CSF, cerebrospinal fluid; FAME, BioFire FilmArray Meningitis/Encephalitis.

<sup>a</sup>Fisher exact test.

<sup>b</sup>The CSF leukocyte count was determined in 324 patients; CSF specimens from 6 patients had too many leukocytes for an exact count to be performed (missing values, n = 6 for FAME negative, n = 1 for routine test positive and n = 5 for routine test negative).

<sup>c</sup>The protein and glucose levels in the CSF were determined in 326 patients; the remaining 4 CSF samples were not measured (missing values, n = 4 for FAME negative and n = 4 for routine test negative).

pathogen detection between conventional diagnostics and FAME. Conversely, the lowest concordance was observed at VT, the center without molecular diagnostics. At the study site VT, of the 8 pathogens covered by FAME, none was detected by conventional diagnostics.

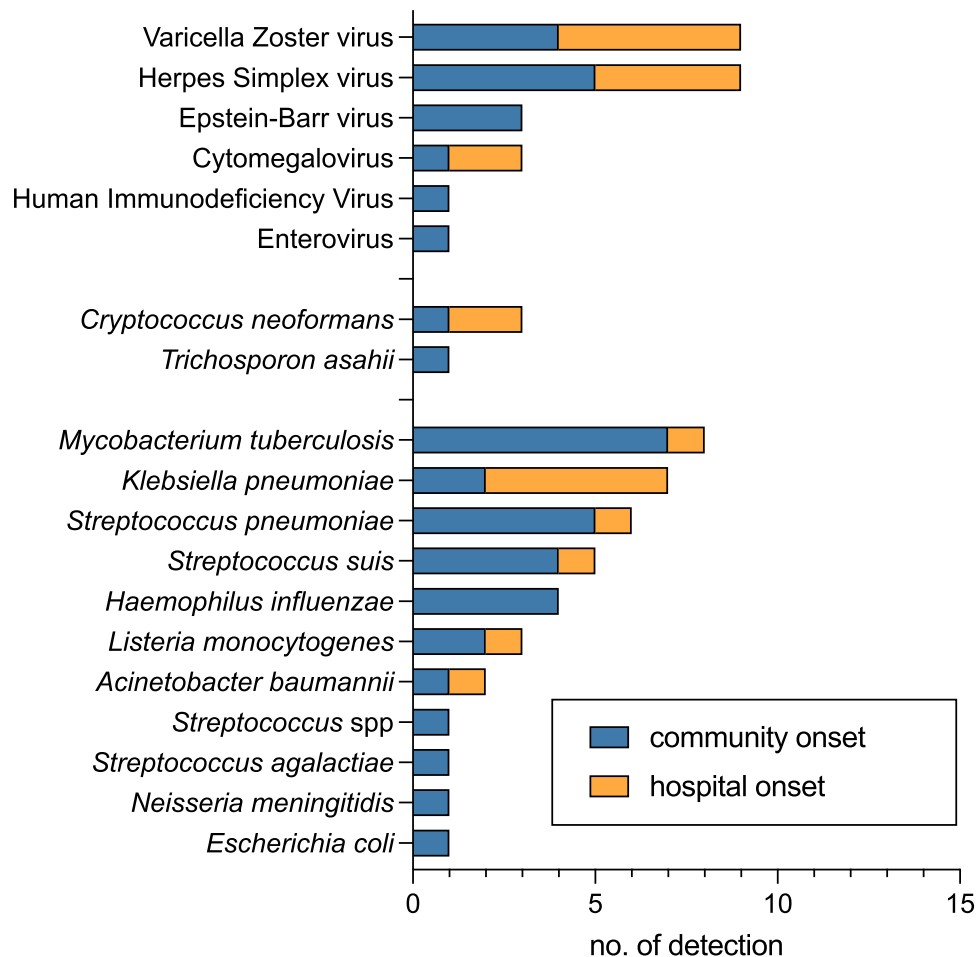
After a thorough review of patient data and charts of discrepancies between FAME results and conventional diagnostic methods, there were 286 matched sample results with an overall match rate of 87% (286/330). In 6 CSF specimens, FAME yielded positive results, primarily for the detection of viral pathogens (3 CMV and 1 HSV-1), as well as for *H influenzae* (n = 2) (Table 2). For the samples with positive CMV and HSV-1 signals in FAME, another pathogen (*K pneumoniae*, *A baumannii*, and *M tuberculosis*) could be detected by blood culture or other molecular methods, which may better explain the clinical presentation. However, no specific PCR was ordered for the remaining two samples with *H influenzae* detection by FAME, so we cannot be sure whether these samples were false positives. In addition, there were 4 pathogens, *E coli* (1/1), *C neoformans* (2/3), HSV-1 (3/8), and VZV (1/9), that were included in the FAME but yielded negative results (Supplementary Tables 2 and 3). Although *M tuberculosis*, *K pneumoniae*, and *S suis* were among the most detected bacterial pathogens, these targets were not included in the FAME panel.

#### Implications for Antimicrobial and Diagnostic Stewardship

The national recommendation for antibiotic treatment regimens for patients with suspected bacterial meningitis is summarized in Supplementary Table 4. The clinical data and the laboratory results were analyzed retrospectively to determine the potential impact on antibiotic prescribing in our study cohort. Of the 33 FAME-positive cases, 20/33 (61%) patients were indicated for inappropriate empirical antibiotic therapy. If the FAME results had been available earlier, 20 patients would have benefited from FAME diagnosis, including antibiotic discontinuation in 8 patients, antibiotic de-escalation in 5, antibiotic change in 3, optimization of therapy in 2, antibiotic de-escalation and antiviral drug discontinuation in 1, and antiviral drug discontinuation in 1 (Supplementary Table 2).

After extensive analysis by machine learning algorithms of various clinical parameters and patient data collected for this study to predict pathogen positivity using either conventional methods or FAME indicated that conventional diagnostic markers emerge as the most important predictors (error rate of 10.61% for FAME and 11.52% for routine diagnostics). Specifically, these markers include CSF cell counts, abnormal (low) glucose levels, and elevated protein levels. Consequently, the integration of complementary and broadly targeted molecular diagnostics for CNS infections has the potential to improve diagnostic accuracy in





**Figure 1.** Distribution of bacterial, viral and fungal pathogens by onset of infection.

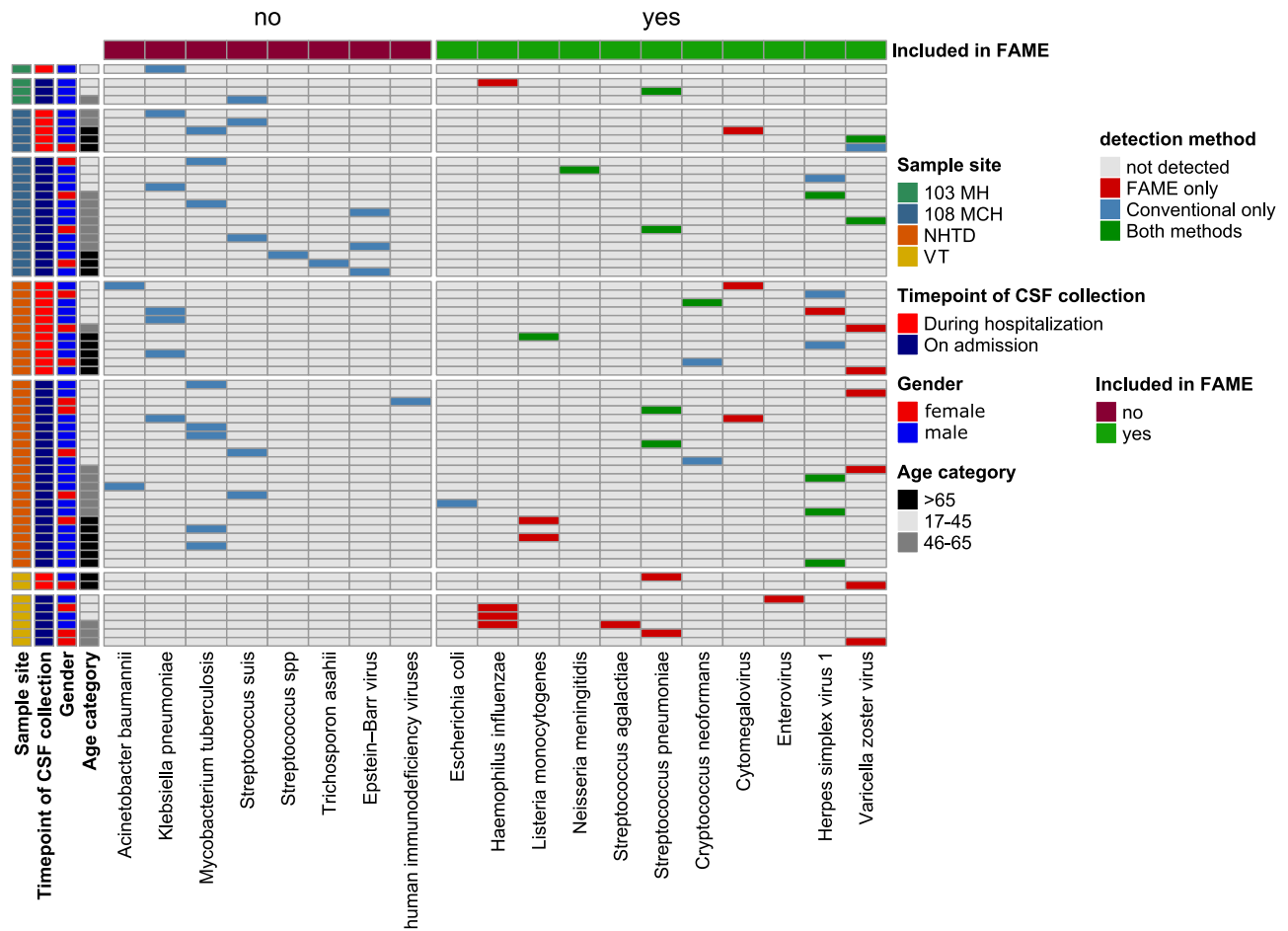
patients with suspected CNS infections who present with these clinical parameters ([Supplementary Figure 2](#)).

## DISCUSSION

Our study showed that targeted molecular diagnostics, such as FAME or in-house molecular diagnostics, can complement conventional culture-based microbiological diagnostics in detecting CNS infections. Furthermore, clinical presentation and laboratory parameters such as headache, neck stiffness, and elevated CSF cell count, protein, and glucose levels can be used to improve the diagnostic algorithm for CNS infections in Vietnam. Implementing an evidence-based diagnostic algorithm in the sense of combined antimicrobial/diagnostic stewardship could increase cost-effectiveness by avoiding overtesting or overordering of expensive molecular tests [11, 12]. A study by Broadhurst et al [13], showed that the positivity rate of FAME could be increased from 11.5% (53/459) to 18.6% (49/263) by implementing a testing algorithm.

Inappropriate administration and/or overuse of antibiotics to patients is one of the main causes of the emergence of resistant bacteria, leading to significantly longer hospital stays and considerable costs for the healthcare system as well as for patients and their families [14]. The implementation of rapid molecular diagnostics, such as FAME, could reduce the turnaround time of microbiological diagnostics of CSF, potentially leading to faster optimization/adjustment of antimicrobial therapy ([Supplementary Table 2](#)). Thus, complementing FAME to antimicrobial stewardship programme interventions may help to enhance clinical impact avoiding the overuse of antimicrobial substances.

Another benefit of implementing molecular diagnostics for diagnosing CSF infection is the possibility of simultaneous detection of bacterial, viral, and fungal pathogens. Moreover, molecular diagnostics have been shown to have a higher sensitivity for patients previously treated with antibiotics before sampling [15]. Diagnostic results should always be interpreted in conjunction with the clinical features, CSF analysis, and other available microbiological results. In our study, FAME contributed to the identification of more than 9 bacteria in culture-



**Figure 2.** Overview of pathogen detection by various diagnostic algorithms. 103 MH, 103 Military Hospital; 108 MCH, 108 Military Central Hospital; NHTD, National Hospital for Tropical Diseases; VT, Viet Tiep Friendship Hospital; FAME, BioFire FilmArray Meningitis/Encephalitis Panel; CSF, cerebrospinal fluid.

negative CSF samples, including *L monocytogenes* (n = 3), *H influenzae* (n = 3), *S pneumoniae* (n = 2), and 1 case of co-detection (*H influenzae* and *S agalactiae*), of which 4 patients had taken antibiotics before lumbar puncture. Potential explanations for the discordance in the detection of *H influenzae* and *S pneumoniae* could be the higher sensitivity of molecular methods than conventional culture [5] or false positive signals of the FAME assay, which has been reported previously [16].

However, we also observed several false-positive and false-negative signals by FAME in this study. After a thorough review of the patient data and charts of the inconsistencies between the results obtained by FAME and conventional diagnostic methods, there were 7 instances in which FAME produced false-negative results for pathogens included in its panel. These included HSV-1 (n = 3), *C neoformans* (n = 2), *E coli* (n = 1), and VZV (n = 1). Notably, patients in these cases had clinical symptoms consistent with a CNS infection, which were supported by the results of conventional diagnostic tests. It is of note that only *E coli* with a K1 capsule type is included in the

FAME PCR panel; non-K1 *E coli* strains will therefore not be detected. Although, *C neoformans* can be detected by the FAME, implementation of *Cryptococcus* antigen testing in addition to microscopy and culture remains an important strategy in the diagnosis and management of cryptococcal disease [17]. In 6 CSF samples, FAME yielded positive results in contrast to the standard-of-care diagnostics, primarily for the detection of viral pathogens (3 CMV and 1 HSV-1), as well as for *H influenzae* (n = 2), primarily for the detection of viral pathogens (3 CMV and 1 HSV-1), as well as for *H influenzae* (n = 2). It is noteworthy that for CMV and HSV-1, microbiologic analysis of CSF and/or blood cultures also revealed positive results for other bacterial pathogens (*CMV/M tuberculosis* n = 1, *HSV/K pneumoniae* n = 1, *CMV/A baumannii* n = 1, and *CMV/K pneumoniae* n = 1), which may provide a more accurate explanation for the observed symptoms. Two cases of *H influenzae* detection were likely to be false-positive signals because CSF analysis showed no pleocytosis, normal protein and glucose levels, and no agreement with the diagnosis at discharge (Supplementary Table 1). The determination of false-positive

**Table 2. Comparison of FAME and Comparator Test Results for the Diagnosis of CNS Infections in Vietnam**

Results Interpretation	FAME	Comparator Methods (CSF Culture and/or PCR)	Clinical Diagnosis	No. of Patients	Identified Pathogens
<b>Concordant results</b>					
Positive	Positive	Positive (concordant pathogen)	Agreement	13	<i>S pneumoniae</i> (n = 4), HSV-1 (n = 4), VZV (n = 2), <i>N meningitidis</i> (n = 1), <i>L monocytogenes</i> (n = 1), <i>C neoformans</i> (n = 1).
Negative	Negative	Negative	Agreement	7	<i>L monocytogenes</i> (n = 3), <i>S pneumoniae</i> (n = 2), <i>H influenzae</i> (n = 1), <i>H influenzae</i> + <i>S agalactiae</i> (n = 1)
...					
<b>Discordant results</b>					
Expected pathogen was included in the FAME panel					
Negative	Negative	Positive (pathogens included in FAME)	Disagreement	7	HSV-1 (n = 3), <i>C neoformans</i> (n = 2), <i>E coli</i> (n = 1), VZV (n = 1)
Positive	Positive	Negative	Disagreement	2	<i>H influenzae</i> (n = 2)
Positive	Positive	Positive (discordant pathogen)	Disagreement	4	CMV/JM tuberculosis (n = 1), HSV-1/K pneumoniae (n = 1), CMV/JA baumannii (n = 1), CMV/K pneumoniae (n = 1)
Not included in the FAME panel or standard-of-care diagnostic panel					
Positive	Not performed/not ordered	NA	NA	7	VZV (n = 6) and Enterovirus (n = 1)
Not included	Positive	Positive	NA	24	<i>M tuberculosis</i> (n = 7), <i>K pneumoniae</i> (n = 5), Epstein-Barr virus (n = 3), <i>A baumannii</i> (n = 1), <i>Trichosporon asahii</i> (n = 1), <i>Streptococcus</i> spp (n = 1), and HIV (n = 1)

Abbreviations: CNS, central nervous system; FAME, BioFire FilmArray Meningitis/Encephalitis; HSV, herpes simplex virus; NA, not applicable; VZV, varicella zoster virus.

rates for *H influenzae* in CSF with FAME has been described previously by Zanella et al [18], in which only 1/17 FAME-positive samples could be confirmed by culture.

Our study highlights the importance of considering local epidemiology when selecting the most appropriate test panel for targeted diagnostics. FAME covers relevant pathogens causing community-acquired CNS infections but does not detect ESKAPE pathogens (*E faecium*, *S aureus*, *K pneumoniae*, *A baumannii*, *P aeruginosa*, and *Enterobacter spp*) that are considered to cause CNS infections in healthcare settings [12, 19]. Importantly, the performance of the panel is expected to depend on the epidemiology of CNS infections in different geographic regions [7]. In Vietnam, CNS infections are complex because they can be caused by a different pathogen spectrum compared to those in high-income and industrialized countries. *M tuberculosis*, which was also not included in the panel, remains a major public health challenge in Vietnam, where the incidence ranges from 260 to 399 per 100 000 population [20]. Meanwhile, *S suis* is one of the most common pathogens causing CNS infections in Vietnam and is associated with the consumption of undercooked pork or raw pig blood [21, 22]. In line with previous studies, our data suggest a lower sensitivity of the FAME assay in detecting HSV-1 and *C neoformans* [23–25] (Supplementary Table 3). Our results suggest that the implementation of a local epidemiology-adapted molecular diagnostic panel may be a better option than a commercial molecular diagnostic platform for resource-limited settings, as demonstrated by the performance of the standard of care diagnostics of the 108 MCH in this study.

In conclusion, our study suggests that the algorithm for effective use of FAME should be applied to patients with acute CNS infections not related to neurosurgery and a CSF pleocytosis of 5 cells/mm<sup>3</sup>. Although FAME is proving invaluable in the rapid detection of common meningitis pathogens, it should be used as a complementary rather than a replacement for conventional testing because it may not detect all pathogens associated with CNS infections. The implementation of FAME in a resource-limited laboratory setting with limited access to molecular methods could improve the diagnostic accuracy. Practitioners must exercise caution in interpreting and selecting results, considering the regional specificity of commercially available targeted molecular diagnostics.

### Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.



## Notes

**Author Contributions.** T.P.V. designed, supervised the study, and contributed to the study materials and assays. D.N. and S.P. were involved in the conceptualization and contributed to the study materials. D.V.D., L.T.L.K., K.S., and V.U. performed the experimental procedures. D.V.D., D.N., S.H., J.R., and S.B. were involved in the statistical analysis and validation of the results. D.V.D., V.V.S., N.D.M., N.X.H., H.X.Q., T.T.L., V.D.T., L.H.S., and N.T.T. recruited the patients and contributed to the investigation materials for sampling procedures. D.V.D. wrote the first draft. T.P.V., D.N., S.H., J.R., P.K., D.V.D., and N.H. reviewed the first draft. All authors have read and approved the manuscript.

**Data availability.** Not Applicable. The authors confirm that the data supporting the findings of this study are available within the article and in its supplementary material.

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**Ethical approval and consent to participate.** Written informed consent was obtained from all hospitalized patients and/or their relatives, and from parents if subjects were aged <18 years. The study protocol was approved by the 108 MCH institutional review board (108MCH/RES/MENTNGITIS-V-D3-25042017). All experiments were conducted in accordance with ICH GCP guidelines and regulations.

**Consent for publication.** All authors read and consented to publish the article.

**Potential conflicts of interest.** All authors: No reported conflicts.

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