International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

A Retrospective Analysis of the BioFire FilmArray Meningitis / Encephalitis Panel in a Tertiary Care Setting

Velamuri Abhishek[1], Sukrutha Gopal Reddy[2]

[1]DNB Resident, Department of Microbiology, KIMS, Secunderabad

[2]Senior Consultant, Department of Molecular Biology, KIMS, Secunderabad
ORCID RECORD: https://orcid.org/0009-0008-1432-4600

Abstract: ***Meningitis and encephalitis (ME) represent critical, life-threatening central nervous system (CNS) emergencies requiring immediate etiological identification to optimize clinical outcomes. Delayed or inaccurate diagnosis prolongs empirical, broad-spectrum antimicrobial use, increasing the risks of drug toxicities and accelerating the development of antimicrobial resistance. Traditional laboratory modalities- including cerebrospinal fluid (CSF) Gram staining, automated aerobic cultures, and single-plex Polymerase Chain Reaction (PCR)- frequently exhibit lengthy turnaround times (TAT) or severely compromised sensitivity, particularly following the pre-hospital administration of empirical antibiotics. This comprehensive retrospective evaluation analyzes the diagnostic yield, pathogen distribution, and antimicrobial stewardship impacts of the multiplex BioFire FilmArray Meningitis/Encephalitis (ME) Panel at a high-volume tertiary healthcare facility in South India. Clinical and molecular data collected from 439 consecutive CSF specimens between January 1, 2022, and May 27, 2026, were analyzed. The assay evaluated 14 specific targets (bacterial, viral, and fungal) simultaneously in approximately one hour. The overall diagnostic yield stood at 12.3% (n = 54/439). Streptococcus pneumoniae and Enterovirus were identified as the predominant bacterial and viral etiologies, respectively. Furthermore, this study demonstrates the panel's capacity to facilitate rapid de-escalation of empirical therapies (e.g., discontinuation of intravenous acyclovir or broad-spectrum carbapenems), while addressing critical implementation challenges such as false-positives from latent viral shedding and the inherent threat of regional off-panel pathogens.***

Keywords: Meningitis Panel, Encephalitis, BioFire Film Array, Cerebrospinal fluid, Antimicrobial Stewardship

1. Introduction

Acute infectious syndromes affecting the central nervous system (CNS), specifically meningitis and encephalitis, remain major contributors to global morbidity, long-term neurological deficits, and mortality. Because the clinical presentations of bacterial, viral, and fungal central nervous system infections overlap significantly (e.g., fever, altered mental status, meningismus, and seizures), empirical administration of broad-spectrum antibiotics and antivirals is standard clinical practice. However, prolonged empirical coverage exposes patients to adverse drug events, inflates hospital costs, and drives selection pressures for multidrug-resistant pathogens.

Historically, laboratory confirmation has relied on conventional methodologies like cerebrospinal fluid (CSF) microscopic examination (Gram stain), biochemical profiling, and automated bacterial/fungal cultures. While culture methods remain crucial for downstream antimicrobial susceptibility testing (AST), their sensitivity drops precipitously (often by more than 50%) if the patient receives even a single dose of antimicrobial therapy prior to the lumbar puncture (LP). Single-plex real-time PCR assays offer high sensitivity but require the clinician to suspect a specific pathogen *a priori*, creating a sequential testing bottleneck that delays definitive diagnosis.

To address these limitations, syndromic multiplex molecular panels have been integrated into critical care pathways. The BioFire FilmArray ME Panel (BioFire Diagnostics, Salt Lake City, UT, USA) is a closed, automated nested multiplex PCR platform that extracts, amplifies, and identifies nucleic acids for 14 primary pathogens directly from 200 µL of uncentrifuged CSF in approximately one hour. The panel targets a specific array of pathogens:

- **Bacterial Targets:** *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Streptococcus agalactiae*, and *Escherichia coli* K1.
- **Viral Targets:** Enterovirus, Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Varicella Zoster Virus (VZV), Human Herpesvirus 6 (HHV-6), Cytomegalovirus (CMV), and Human Parechovirus.
- **Fungal Targets:** *Cryptococcus neoformans/gattii*.

While the analytical performance of this panel is well-established, its real-world clinical utility, cost-effectiveness, and impact on antimicrobial stewardship programs (ASP) vary substantially across different geographical regions and hospital ecosystems. This variation is especially pronounced in resource-constrained or high-burden settings where empirical antibiotic abuse is common and regional pathogens not covered by the panel are endemic. This study evaluates the diagnostic yield, epidemiological profile, and real-world clinical impact of the BioFire ME panel within a major Indian tertiary referral center.

2. Materials and Methods

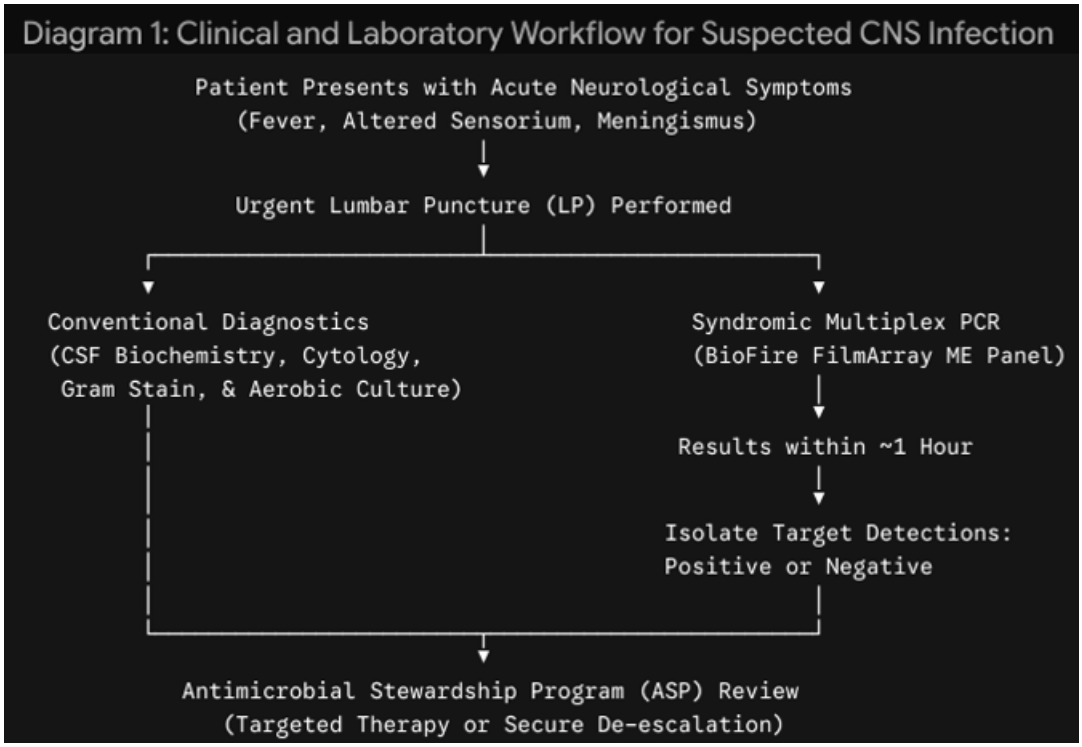
Study Design and Clinical Setting

A retrospective, observational study was conducted at the Krishna Institute of Medical Sciences (KIMS), Secunderabad, India, a multi-specialty tertiary care hospital.

Electronic health records (EHR) and laboratory information system (LIS) databases were systematically reviewed for all patients who underwent CSF evaluation via the BioFire FilmArray ME Panel between **January 1, 2022, and May 27, 2026**.

Inclusion and Exclusion Criteria

- **Inclusion Criteria:** All consecutive patients (neonatal, pediatric, and adult) presenting with clinical features highly suggestive of acute CNS infection- defined as the acute onset of fever accompanied by headache, altered sensorium, focal neurological deficits, signs of meningeal irritation, or new-onset seizures—who underwent a lumbar puncture and subsequent multiplex molecular testing.
- **Exclusion Criteria:** CSF samples obtained from external facilities that did not maintain a documented cold chain, or specimens with insufficient volume (<200 µL) for the complete run. Repeat tests for the same disease episode were excluded from diagnostic yield calculations to avoid artificial skewing.



Laboratory Processing and Assay Mechanism

CSF samples were collected in sterile tubes and processed immediately. The BioFire FilmArray ME Panel was performed according to the manufacturer's operational instructions. The reagent pouch was hydrated, and 200 µL of uncentrifuged CSF was injected along with the provided sample buffer. The automated system executes cell lysis, nucleic acid purification, a first-stage multiplex reverse transcription PCR (RT-PCR), and a second-stage nested single-plex PCR to detect the specific amplicon targets.

Concurrently, traditional diagnostic pathways were executed:

1) **Cytochemical Analysis:** Total leukocyte count (WCC), differential cell count, glucose level, and total protein concentration using automated analysers.
2) **Microbiology:** Gram staining and direct microscopic visualisation, followed by inoculation onto sheep blood agar, chocolate agar, and MacConkey agar plates, incubated aerobically and under 5% CO_2 for up to 5 days.
3) **Fungal Surveillance:** Inoculation onto Sabouraud Dextrose Agar (SDA) and India ink preparation when clinically indicated.

Data Extraction and Stewardship Metrics

The collected clinical parameters included baseline demographics, prior exposure to antimicrobials before the lumbar puncture, and specific times of lab registration, result verification, and clinical actioning. The primary antimicrobial stewardship metrics were defined as:

- The clinical turnaround time (cTAT): measured from sample receipt in the lab to result entry in the EHR.
- The exact duration of empirical intravenous acyclovir therapy in patients with suspected viral encephalitis.

Statistical Analysis

Continuous variables were expressed as medians with interquartile ranges (IQR) or standard deviations, while categorical variables were presented as absolute counts and percentages. Sensitivities and specificities were calculated using conventional culture as the comparative benchmark for bacterial pathogens. Differences between categorical metrics were verified utilizing the Pearson Chi-Square test (χ^2) or Fisher's exact test where cell counts dropped below 5, with significance set at $P < 0.05.

3. Results

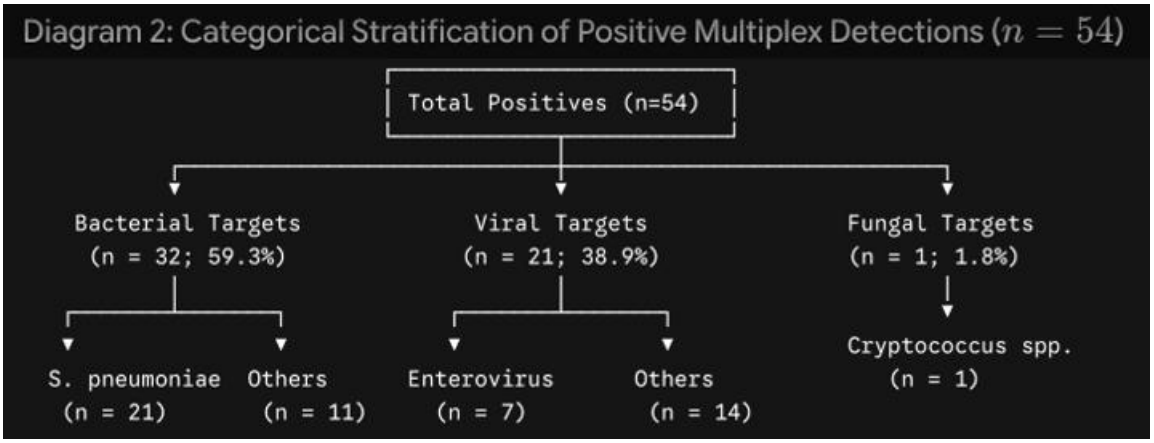
Cohort Demographics and Baseline CSF Profiles

During the study period, **439 distinct CSF specimens** from unique symptomatic patients met all inclusion parameters. The median age of the cohort was 39.5 years (IQR: 14–61 years); 56.7% (n = 249) were male. Prior empirical

antimicrobial exposure before the lumbar puncture was documented in 41.2% (n = 181) of the total population.

Diagnostic Yield and Pathogen Distribution

Out of 439 samples analyzed, the multiplex panel detected at least one pathogen in **54 samples**, demonstrating an overall diagnostic yield of **12.3%**. The remaining 385 samples (87.7%) returned negative results for all 14 panel targets.



Bacterial pathogens comprised 59.3% (n=32) of all positive cases, viral pathogens accounted for 38.9% (n=21), and fungal identification was limited to a single case of *Cryptococcus neoformans/gattii* (1.8%). No polymicrobial or dual-pathogen co-infections were detected in this cohort.

Specific Pathogen Identification Metrics

Streptococcus pneumoniae was the most common pathogen identified overall (n = 21), accounting for 38.9% of all positive results and 65.6% of all bacterial detections. Among viral targets, *Enterovirus* was the most frequent (n = 7), followed by *Varicella Zoster Virus* (n = 6) and *Herpes Simplex Virus 1* (n = 5).

Table 1 provides a detailed breakdown of the qualitative results obtained from the BioFire FilmArray ME Panel.

Table 1: Pathogen Recovery Profile and Comparative Culture Positivity

Target Pathogen Class	Specific Microorganism Target	BioFire Positive Count (n)	% of Total Positives	Concurrent Culture Positive (n)
Bacterial	*Streptococcus pneumoniae*	21	38.90%	9
	Haemophilus influenzae	5	9.30%	1
	Listeria monocytogenes	3	5.60%	1
	Streptococcus agalactiae	2	3.70%	2
	Neisseria meningitidis	1	1.90%	0
	Escherichia coli K1	0	0.00%	0
Viral	Enterovirus	7	13.00%	N/A
	Varicella Zoster Virus (VZV)	6	11.10%	N/A
	Herpes Simplex Virus 1 (HSV-1)	5	9.30%	N/A
	Human Herpesvirus 6 (HHV-6)	2	3.70%	N/A
	Cytomegalovirus (CMV)	1	1.90%	N/A
	Herpes Simplex Virus 2 (HSV-2)	0	0.00%	N/A
	Human Parechovirus	0	0.00%	N/A
Fungal	*Cryptococcus neoformans/gattii*	1	1.90%	1
Total	**All Pathogens Combined**	54	100.00%	14

Comparison with Conventional Culture

Conventional bacterial cultures yielded positive results in only 13 cases, plus 1 positive fungal culture, totaling **14 culture-positive samples** across the 439-sample cohort. In contrast, the molecular panel detected 32 bacterial cases and 1 fungal case (n=33). Notably, 19 bacterial cases identified by molecular methods remained culture-negative. Review of clinical histories indicated that 84.2% (n = 16) of these 19 patients had received broad-spectrum antibiotics (such as ceftriaxone or meropenem) before the lumbar puncture was performed.

The overall analytical concordance for the bacterial targets showed a specificity of **99.1%**, while its diagnostic yield for bacterial targets was significantly higher than that of traditional culture methods (7.3% vs 3.0%, P < 0.001). Conversely, conventional cultures identified two bacterial pathogens that were not detected by the molecular assay: one *Acinetobacter baumannii* isolate and one *Klebsiella pneumoniae* isolate, both obtained from post-neurosurgical patients with healthcare-associated ventriculitis. Neither organism is included in the BioFire ME Panel target menu.

Antimicrobial Stewardship and Operational Efficiency

The implementation of the automated multiplex panel led to a significant reduction in the laboratory clinical turnaround time (cTAT). The median processing time from lab log-in to result verification was **1.1 hours** (IQR: 0.9–1.4 hours), compared to **44.5 hours** (IQR: 24.0–72.0 hours) required to finalise conventional negative culture profiles (P < 0.0001).

This rapid turnaround directly influenced clinical decision-making:

- **Empirical Antiviral Cessation:** For the 385 patients who tested negative on the multiplex panel, empirical intravenous acyclovir- initiated at admission for suspected herpes simplex encephalitis—was discontinued within a median of **4.2 hours** after receipt of the molecular report, provided that clinical findings and baseline CSF white cell counts supported a low probability of viral encephalitis.
- **De-escalation of Broad-Spectrum Antibiotics:** In 14 cases where *Streptococcus pneumoniae* or *Haemophilus influenzae* was rapidly detected, clinicians safely de-escalated therapy from broad-spectrum carbapenems (e.g., Meropenem) to narrow-spectrum targeted regimens (e.g., Ceftriaxone or Penicillin G) once susceptibility profiles were confirmed by traditional microbiology.

4. Discussion

This single-centre retrospective analysis demonstrates that integrating syndromic multiplex PCR testing into clinical workflows significantly improves the management of suspected CNS infections in a tertiary facility. The observed diagnostic yield of 12.3% aligns with rates reported in major international validation studies, which typically range between 8% and 23% depending on institutional testing criteria.

One of the most valuable aspects of multiplex molecular testing is its ability to rescue diagnostic yields in patients who have already received antibiotics. In resource-rich and resource-limited settings alike, clinicians frequently administer empirical antimicrobials prior to performing a lumbar puncture to avoid delaying treatment during critical therapeutic windows. While this practice is clinically necessary, it often renders subsequent CSF cultures sterile. In this study, 19 bacterial cases were identified by molecular methods but remained completely negative on culture. The majority of these patients had documented pre-treatment with cephalosporins or carbapenems. Because PCR detects highly stable microbial DNA rather than relying on viable, replicating organisms, it helps bypass the limitations associated with pre-hospital antibiotic exposure, providing crucial etiological clarity.

The pathogen distribution observed in this cohort reveals a high prevalence of *Streptococcus pneumoniae* (n=21), highlighting a significant burden of pneumococcal disease within this regional population. In contrast, Western validation cohorts often report higher relative frequencies of viral pathogens, such as Enteroviruses or HSV-1, over bacterial isolates. This epidemiological variation underscores the importance of interpreting syndromic multiplex assays within the context of local disease prevalence and regional vaccination histories.

5. Diagnostic Limitations

The Clinical Dilemma of Latent and Asymptomatic Viral Detections

A primary concern with highly sensitive molecular platforms is the risk of detecting latent viruses that may not be the primary cause of the patient's acute illness. For example, human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV) can establish lifelong latency within central nervous system tissues or mononuclear cells. Their detection may reflect low-level asymptomatic reactivation triggered by adjacent neuro-inflammatory stress or chromosomal integration rather than an active, primary infectious process. Therefore, a positive molecular result for these targets must always be interpreted in conjunction with quantitative viral load testing, clinical presentation, and baseline CSF cytochemical parameters (e.g., white blood cell counts and protein levels) to avoid inappropriate or prolonged antiviral therapy.

The Risk of Off-Panel Pathogens

A negative result on the syndromic panel does not rule out a central nervous system infection. In India, several critically important pathogens are not included in the BioFire ME Panel's 14-target menu:

- *Mycobacterium tuberculosis* (the causative agent of tubercular meningitis, a major cause of morbidity in developing countries).
- Arboviruses, such as Japanese Encephalitis Virus (JEV), Dengue, and Chikungunya.
- Nosocomial pathogens, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and various Enterobacteriaceae species.

This coverage gap was highlighted in this study by the isolation of *Acinetobacter baumannii* and *Klebsiella pneumoniae* from two post-neurosurgical patients whose molecular panels returned completely negative results. Consequently, relying solely on multiplex assays can create a false sense of security, potentially delaying the diagnosis of off-panel or healthcare-associated infections.

6. Recommendations for Diagnostic Stewardship

To optimise clinical utility and manage healthcare costs, healthcare facilities should implement structured diagnostic stewardship protocols rather than allowing unrestricted clinician ordering. Implementing strict laboratory gating criteria- such as restricting automated multiplex PCR testing to CSF specimens with an elevated leukocyte count (e.g., WCC > 10 - 50 cells/µL) or abnormal biochemistry—can significantly reduce unnecessary testing while increasing the pre-test probability and the overall positivity rate. Exceptions to these gating rules should be clearly defined, such as granting immediate access for severely immunocompromised individuals or neonates, who may fail to mount a robust cellular response in the early stages of a central nervous system infection.

7. Conclusion

The BioFire FilmArray ME Panel is a valuable diagnostic tool within tertiary care settings, offering rapid and precise pathogen identification that supports effective antimicrobial stewardship and timely clinical decision-making. Its ability to detect microbial nucleic acids in patients who have already received antibiotics significantly reduces the diagnostic limitations of conventional culture methods. However, due to its inability to detect regional off-panel pathogens like *Mycobacterium tuberculosis* or common nosocomial

organisms, it cannot entirely replace traditional culture techniques, microscopic evaluation, or clinical assessment. Optimal use of this technology requires its integration into structured diagnostic stewardship frameworks that combine rapid molecular data with established clinical and cytochemical criteria.

References

[1] Morbani D, Alifoni A, Di Do M, Loffredo M, M T I, Rizzato L, et al. Utility of the FilmArray Meningitis/Encephalitis Panel in a Tertiary Care Hospital. *Frontiers in Neurology*. 2019 Jun 25; 10: 281.
[2] Buchan BW, Reymann MK, Lindquist D, et al. Clinical Evaluation of a Multiplex PCR Panel for Molecular Diagnosis of Meningitis and Encephalitis. *Journal of Clinical Microbiology*. 2018;56(11): e01235-20.
[3] Schmutz R, Zer F, Nehrbass B, Sulz U, Barennes H. Impact of Syndromic PCR Panels on Diagnosis and Decision Pathways in CNS Infections. *BMC Infectious Diseases*. 2024 Apr 17;24(1):378.
[4] Subedi H, Myint T, Sa W. Real-World Stewardship Impact of Multiplex Molecular Testing for Meningitis: Four Key Opinions. *European Medical Journal*. 2023 Oct;7(3):57–64.
[5] Goenka S. Clinical Utility of the BioFire Meningitis/Encephalitis (ME) Panel in Diagnosing Central Nervous System Infections. *Journal of Medical Microbiology and Infectious Diseases*. 2025 Dec 12; 13(4):185–192.
[6] Ibrahim NH. Efficient use of the BioFire® FilmArray® Meningitis/Encephalitis Panel: a diagnostic stewardship approach. *Internal Medicine Journal*. 2026 Mar 13;56(3):311–318.
[7] Erinmez M. Real-World Evaluation of the BioFire FilmArray ME Panel: Diagnostic Accuracy and Clinical Utility. *Journal of Clinical Virology*. 2024; 172: 105-112.
[8] Niyas VKM, Sasidharan A, Chandran S, Chandran S. Clinical Performance of FilmArray Meningitis/Encephalitis Multiplex Polymerase Chain Reaction Panel in Central Nervous System Infections. *Indian Journal of Critical Care Medicine*. 2022;26(1):67–70.
[9] Erba A, Franzeck CC, Hinic V, Egli A, Osthoff M. Utilization of a Meningitis/Encephalitis PCR panel at the University Hospital Basel – a retrospective study to develop a diagnostic decision rule. *Frontiers in Medicine*. 2024 Apr 17; 11: 1351903.
[10] Rafiei N. Clinical and cost implications of Biofire FilmArray® meningitis / encephalitis panel testing. *Journal of Hospital Infection*. 2025 Mar 22; 145: 72–81.
[11] Rasti R. Clinical utility of the FilmArray® meningitis/encephalitis panel in children with suspected central nervous system infection in a low-resource setting – a prospective study in Southwestern Uganda. *BMC Infectious Diseases*. 2025 Mar 22;25(1):412.
[12] Myint T. Comparison of positive BioFire FilmArray meningitis/encephalitis (ME) panels, CSF cultures, CSF parameters, clinical presentation and in-patient mortality among patients with bacterial and fungal meningitis. *Microbiology Spectrum*. 2024 Dec 23;12(6):e00014-24.
[13] Thirunavukkarasu S, Gupta S, Chari C, et al. Syndromic Molecular Testing of Meningitis in Indian Intensive Care Units: Epidemiology and Stewardship Outcomes. *Indian Journal of Critical Care Medicine*. 2022 Dec;26(12):1233–1240.
[14] Prakash V, Agrawal A, Chaudhry A, Kholia P, Dhingra R. Molecular Epidemiology of Infectious Meningitis/Encephalitis Over One Year at a Tertiary Center in Northern India. *Journal of Laboratory Physicians*. 2023;15(4):45–52.
[15] Singh K, Sheikh A, Hassan M. Unsolved Challenges in Interpreting Multiplex PCR Panels for Central Nervous System Infections. *Clinical Infectious Diseases*. 2021;19(8):811–818.
[16] Hanson KE, Slechta ES, Killgore KM, et al. Pretest Probability and Gating Strategies to Optimize Multiplex PCR Testing for Central Nervous System Syndromes. *Journal of Clinical Microbiology*. 2020;58(4):e01822-19.
[17] Moffa M, Patel R, Broadhurst MK, et al. Impact of Laboratory-Gated Multiplex Molecular Testing for Meningitis on Hospital Length of Stay and Antibiotic Expenditure. *Diagnostic Microbiology and Infectious Disease*. 2021;99(2):115214.
[18] Liesman RM, Strasburg DM, Heitman AK, et al. Evaluation of a Commercial Multiplex Molecular Panel for Diagnosis of Infectious Meningitis and Encephalitis. *Journal of Clinical Microbiology*. 2018;56(4):e01923-17.
[19] Binnicker MJ. Multiplex Molecular Panels for Central Nervous System Infections: Three Pillars of Diagnostic Stewardship. *Clinics in Laboratory Medicine*. 2020;40(4):453–463.
[20] Venkatesan A, Tunkel AR, Bloch KC, et al. Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis: Consensus Statement of the International Encephalitis Consortium. *Clinical Infectious Diseases*. 2013;57(8):1114–1128.