

VIRAL MENINGOENCEPHALITIS: A REVIEW ON ETIOLOGICAL SPECTRUM & ADVANCES IN LABORATORY DIAGNOSIS

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ABSTRACT

Viral meningoencephalitis remains a critical global health priority, representing a significant burden of neurological disease and mortality worldwide. Characterised by acute inflammation of the meninges and brain parenchyma, this disease demands rapid diagnostic precision to guide clinical intervention and effective outbreak response. This review aims to provide a comprehensive analysis of viral aetiologies, mapping the circulating spectrum of common pathogens and discussing various advances in laboratory diagnosis. Analysis shows that common worldwide pathogens include herpes simplex virus (HSV) and enteroviruses. Nevertheless, Japanese encephalitis (JE) and Nipah virus remain persistent public health threats in Asian countries, including Malaysia. Furthermore, this review compares traditional laboratory methods with molecular diagnostic approaches to establish diagnostic protocols. While traditional laboratory methods which include cerebrospinal fluid analysis, viral culture and serology provide a reliable baseline, they are often hindered by limited sensitivity. In contrast, advanced platforms such as multiplex PCR and next-generation sequencing (NGS) offer rapid, high-sensitivity detection of both known and emerging pathogens. However, the transition to these technologies is often limited by high costs and technical complexity in low-resource settings. By synthesising these issues, this review contributes to scientific knowledge dissemination as well as prioritises evidence-based literature to ensure that the rapid detection capabilities of genomic platforms are utilised effectively alongside accessible conventional tools. Ultimately, this review serves as a guide for clinicians and public health authorities to enhance the laboratory diagnosis of viral meningoencephalitis in an evolving infectious landscape.

KEYWORDS: Viral meningoencephalitis, herpes simplex virus (HSV), enterovirus, viral PCR, next-generation sequencing (NGS)

INTRODUCTION

Viral meningoencephalitis, a condition characterised by inflammation of both the meninges and brain parenchyma, remains a major neurological emergency with potentially devastating consequences (1). Meningitis is a medical condition characterised by inflammation of the meninges, which are the protective membranes that surround the brain and spinal cord (2). This specific definition of meningitis is crucial, as it distinguishes this condition from other neurological diseases (3). Importantly, meningitis does not involve the brain parenchyma itself, which is the functional tissue of the brain consisting of neurons and glial cells (4). Furthermore, the definition overtly excludes various types of inflammation affecting the spinal cord (myelitis) (2). These distinctions are crucial for accurate diagnosis and appropriate medical treatment, as different conditions require different approaches.

Encephalitis refers to an inflammatory disorder affecting the brain parenchyma, accompanied by clinical signs of brain dysfunction (5). The causes can be either non-infective, such as acute disseminated encephalomyelitis (ADEM), or infective, most commonly resulting from a diffuse viral cause (6). Viral encephalitis affects 3.5 to 7.5 per 100,000 individuals, with higher rates observed in younger and older populations (7, 8). The prevalence of specific viral causes of encephalitis has evolved over time. For instance, immunisations show reduced in the incidence of encephalitis caused by mumps and measles (9). Conversely, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) encephalitis are now more commonly reported, particularly in immunocompromised patients, including those with acquired immunodeficiency syndrome (AIDS), organ transplant recipients and individuals undergoing chemotherapy (9).

Viral meningitis which refers to inflammation of meninges following viral infections has contributed to 25,000 to 50,000 hospitalisations in the United States annually (10). In temperate regions, viral meningitis is observed monthly, with a peak incidence of 1 per 100,000 persons (11). However, many viral meningitis cases go undiagnosed as it is a self-limiting condition that often resolves without symptoms (12). Particularly, reported viral meningitis cases are highest during summer and early fall (11). Malaysia, being located in the equatorial region with high biodiversity and close human-animal interactions, has reported several viral encephalitis outbreaks in early years which involve Japanese encephalitis (JE) and Nipah virus outbreak around 1998-1999 that caused morbidity and mortality burdens to the country with up to 40% fatality reported (13,14,15).

Viral meningoencephalitis is a significant cause of central nervous system (CNS) infections, leading to substantial morbidity and mortality worldwide (11,12). The disease can be caused by a wide range of neurotropic viruses, including enteroviruses, herpesviruses, and arboviruses (1,4,7,9,10). However, the etiological agents often remain unidentified in many clinical cases due to limitations in traditional diagnostic methods, resulting in delayed diagnosis and inappropriate clinical management.

Early and accurate identification of the causative viral pathogen is essential for timely patient management, appropriate antiviral therapy and improved clinical outcomes (4). Traditional laboratory diagnostic approaches, such as viral culture and serological assays, are often time-consuming and may have limited sensitivity, particularly during the early stage of infection (16). Advances in molecular diagnostic techniques, including polymerase chain reaction (PCR) and multiplex molecular assays, have significantly improved the ability to detect viral pathogens rapidly and with higher sensitivity and specificity (17).

Despite these technological advances, there is still limited local data on the spectrum of viral pathogens responsible for meningoencephalitis, particularly in many developing regions. Understanding the circulating viral aetiologies is important for improving diagnostic strategies, guiding clinical management and strengthening public health surveillance. Therefore, this review aims to explore the etiological spectrum of viral meningoencephalitis and evaluate advanced laboratory diagnostic methods that ensure rapid pathogen detection, ultimately facilitating effective patient management.

MATERIALS AND METHODS

We searched PUBMED, MEDLINE (National Library of Medicine) and Google Scholar for relevant literature from 1999 to September 2025. The search terms selected were as follows: 'viral meningoencephalitis', 'herpes simplex virus (HSV)', 'enterovirus', 'viral PCR', 'next-generation sequencing (NGS)'. The articles studied are currently published and the criteria included in this paper are: 1) the disease studied is viral meningoencephalitis; 2) the subjects are human; 3) diagnostic techniques of etiological agents; and 4) English writing papers. Meanwhile, exclusion criteria were: 1) non-human studies; 2) non-English articles; 3) treatment of viral meningoencephalitis; 4) animal studies; and 5) no diagnostic evaluation. Review articles and book chapters were also included if considered to provide comprehensive reviews of the topic. The included 72 articles were perused and summarized, and the retrieved content was used for descriptive analysis. A narrative presentation followed the compilation of these data. Ethical approval was not sought as only published data were used for this review.

RESULTS AND DISCUSSION

ETIOLOGICAL SPECTRUM OF VIRAL MENINGOENCEPHALITIS

Worldwide, viruses signify the most common infectious cause of encephalitis, with herpes simplex virus (HSV) and enteroviruses consistently among the leading culprits (18). While the etiological spectrum of viral meningoencephalitis contrasts geographically, for example Southeast Asia, tropical and subtropical regions, the burden of disease is significantly predisposed by seasonal viruses like JE, enteroviruses, dengue virus and emerging zoonotic viruses such as Nipah virus (13,14,19,20).

Under the *Herpesviridae* family, The HSV, particularly HSV-1, is the most common cause of sporadic fatal viral encephalitis in adults in developed countries (21). HSV-1 encephalitis classically affects the temporal lobes, presenting with altered mental status, seizures and focal neurological deficits (22). HSV-2 on the other hand is more commonly affecting neonates and immunocompromised individuals especially related to meningitis (23). CMV causes meningoencephalitis primarily in immunocompromised hosts, such as transplant recipients or individuals with advanced HIV infection, in which symptoms are often non-specific and may include encephalopathy, cranial nerve palsies and ventriculitis (21).

Under the *Flaviviridae* family, JE is a major cause of viral encephalitis in Asia, including Malaysia (24). It is transmitted by *Culex* mosquitoes and preserved in a zoonotic cycle concerning pigs and waterbirds (24). Mortality rates can be as high as 30%, and 30–50% of survivors have long-term neurological sequelae (20). Dengue encephalitis, resulting from the direct invasion of the CNS, has also emerged as a critical neurological complication in hyperendemic regions (25). It is increasingly recognised as a primary contributor to the morbidity associated with severe dengue (25). On the other hand, Zika virus (ZIKV) has been identified as an important cause of congenital viral encephalitis (26). Following its

widespread emergence in South America, ZIKV became globally recognised for its ability to cross the placental barrier and directly infect foetal neural progenitor cells (26).

Meanwhile, enterovirus A71 (EV-A71) and several other non-polio enteroviruses from the *Picornaviridae* family are significant causes of aseptic meningitis and brainstem encephalitis, especially in young children (27). In Malaysia, EV-A71 outbreaks have been associated to severe neurological complications, including cardiopulmonary collapse and death (21). Echoviruses especially echovirus 11, are notably associated with neonatal outbreaks characterised by meningoencephalitis, and often complicated by multi-organ failure and high mortality (28).

A formidable member of the *Paramyxoviridae* family, Nipah virus causes a devastating form of acute febrile encephalitis (29). Its clinical progression is notoriously aggressive, marked by a rapid loss of consciousness, severe brainstem dysfunction and high mortality (30). Imaging and autopsy studies frequently expose multiple microinfarcts and necrotising vasculitis in the brain (31). Measles virus, another paramyxovirus, may cause acute encephalitis, post-infectious encephalomyelitis or subacute sclerosing panencephalitis (SSPE). Although the incidence is rare now due to vaccination, measles-associated encephalitis still arises in areas with low immunisation coverage (32).

This review focuses on summarising these pathogens alongside their diagnostic strategies. As shown in Table 1, this includes primary and alternative diagnostic methods, comprising both traditional and advanced approaches.

Table 1. Overview of Diagnostic Strategies for Viral Meningoencephalitis.

Pathogen	Clinical Significance	Primary Diagnostic	Alternative / Secondary Method	Reference
Herpes Simplex Virus (HSV-1 & 2)	Most common cause of sporadic fatal viral encephalitis in adults.	Cerebrospinal fluid (CSF) PCR.	Magnetic Resonance Imaging (MRI) (temporal lobe involvement) or Brain Biopsy.	7,23,54,55
Enteroviruses (EV-A71, Echoviruses)	Significant causes of aseptic meningitis and brainstem encephalitis in children.	CSF PCR.	Faeces and throat swab PCR/ serology	1,2,27,55
Varicella-Zoster Virus (VZV)	Causes encephalitis or meningitis, especially in elderly or immunocompromised populations.	CSF PCR.	Skin vesicle PCR or Clotted blood (IgM).	8,10,21,38
CMV	Primarily affecting in immunocompromised hosts	CSF PCR.	Blood PCR/ serology	4,6,9,37

JE Virus	Major cause of viral encephalitis in Asia and Malaysia; high mortality and long-term sequelae.	CSF/Serum IgM (Serology).	Multiplex PCR or RT-PCR.	13,19,20,24,35
Dengue Virus	Endemic in Malaysia; increasingly linked with neurological manifestations like ADEM.	RT-PCR (Serum/Plasma)	Serology (NS1-ag/IgM/IgG).	2,17,20,25,33
Nipah Virus	Emerging paramyxovirus causing acute febrile encephalitis with high mortality (~40%).	RT-PCR (CSF/Tissue/Serum/Plasma).	MRI (hyperintense lesions in white matter/brainstem).	14,15,29,30,31
Human Immunodeficiency Virus (HIV)	Rarely causes HIV-related meningoencephalitis, HIV-associated neurocognitive disorders (HAND), and HIV encephalitis	Serum PCR	CSF HIV Viral Load, Serology	5,12,16
Influenza viruses	Causes rare but serious CNS complications involving encephalitis and encephalopathy	Respiratory PCR (Nasopharyngeal (NP) Swab/Aspirate)	Serology	3,11,18,38
Measles Virus	Causes acute encephalitis, post-infectious encephalomyelitis or subacute sclerosing panencephalitis (SSPE).	Serum and CSF Serology (IgM/IgG)	RT-PCR (NP swabs or aspirates/Urine)	12,18,32,47
Novel/Rare Pathogens	Pathogens not covered in standard panels or emerging in specific populations.	Metagenomic NGS (mNGS).	Targeted Sequencing (tNGS).	61,62,63,64,65,66

TRADITIONAL LABORATORY DIAGNOSIS

One of the most essential diagnostic tools in this process is CSF analysis, which aids in distinguishing viral from bacterial causes and guides clinical management (33). However, lumbar puncture which is the primary method of obtaining CSF, is commonly delayed due to the safety use of neuroimaging to rule out raised intracranial pressure (34). In clinical practice, differentiating viral meningoencephalitis from other causes of acute encephalopathy, such as bacterial meningitis, autoimmune encephalitis or metabolic encephalopathies, can be challenging, predominantly in settings with limited access to neuroimaging and laboratory diagnostics (35). Viral culture has traditionally been considered a gold standard for diagnosing viral meningoencephalitis, as it permits for the direct isolation of the causative virus from clinical specimens (36). However, its use has reduced due to the time-consuming nature and lower sensitivity in

contrast to molecular methods (37). Furthermore, CSF viral culture has low sensitivity, hence not commonly accomplished in routine clinical practice, in contrast to bacterial cause of infection (38).

Serological testing remains an important diagnostic tool in the identification of viral meningoencephalitis, predominantly when viral nucleic acids are undetectable by PCR (39). However, preliminary antiviral therapy can overturn the antibody response, possibly leading to false negatives (40). Serologic assays are predominantly important in diagnosing infections caused by arboviruses such as JE virus or the West Nile virus (WNV), where PCR frequently lacks sensitivity due to low viral load in CSF (41). In these cases, diagnosis relies on measuring virus-specific IgM in the CSF and serum, by which detecting IgM in the CSF supports a neuroinvasive process, while comparing paired serum samples confirms that the infection is recent (42).

Despite its value, serological testing is limited by delayed antibody production, cross-reactivity and variable specificity in early stages of illness. As a result, most guidelines recommend early PCR testing; however, if these preliminary results are inconclusive, serological testing is recommended 10 to 14 days after symptoms begin (43). Furthermore, Schibler et al. (2019) highlight the use of paired serum and CSF samples to confirm intrathecal antibody synthesis, refining specificity and reducing false positives due to passive transfer of antibodies from blood (44). Recent European studies accentuate the importance of incorporating serological tests with neuroimaging and molecular techniques to improve the diagnostic precision for viral meningoencephalitis (45).

This approach is particularly valuable in immunocompetent adults, where delayed or partial immune responses can affect diagnostic windows. Neuroimaging, mainly CT and MRI, are significant tools for identifying brain anomalies in patients with viral meningoencephalitis (46). CT scans are beneficial by showing signs of infection such as calcifications or oedematous brain parenchyma, also for ruling out other emergencies like brain bleeding or tumours, however may miss indirect or early signs of encephalitis because it has lower sensitivity compared to MRI, although are usually attained first because they are fast and broadly available (45,46).

ADVANCES IN LABORATORY DIAGNOSIS

Nucleic acid amplification test (NAAT)

The diagnosis of viral meningoencephalitis has progressed significantly with the initiation of advanced molecular methods, which have addressed many restrictions of traditional culture and serological approaches (47). Over the past few decades, nucleic acid amplification test (NAAT), with PCR at the forefront, has fundamentally converted the diagnostic landscape of CNS infections, mostly those of viral origin (48). Molecular diagnostics proposed rapidity, increased sensitivity and the capability to detect viruses directly from CSF or brain tissue, critical for timely therapeutic involvement in this potentially lethal condition (49). PCR can be applied to diagnose any condition where nucleic acids, such as DNA and RNA, or their expression as mRNA, are involved (47, 49,50). PCR is broadly employed to detect both DNA and RNA viruses in CSF (48). NAAT incorporate various techniques, including quantitative PCR and reverse transcription PCR (RT-PCR), have seen increasing application in the analysis of clinical samples, offering both sensitivity and specificity in the recognition of viral nucleic acids (49). Emerging evidence from several studies suggests that the quantification of viral load such as CMV and EBV, expressed as nucleic acid copy number, may assist as a valuable biomarker for evaluating disease severity or predicting clinical outcomes, predominantly among immunocompromised people (47,48,49).

Although traditional PCR can be adapted for quantitative analysis such as by running concurrent standard curves, real-time quantitative PCR (qPCR) has largely replaced it in clinical diagnostics due to its superior accuracy and efficiency in measuring viral load (50). These techniques use reagents capable of constructing fluorescence during the amplification process, allowing real-time monitoring of product accumulation (46).

The intensity of the fluorescent signal, which associates with the initial quantity of target nucleic acid, is measured at each cycle, thus allowing for precise quantification (51). In probe-based qPCR assays, fluorescent reporter molecules are designed to rise in signal proportionally with the amplification of the target sequence, providing both specificity and dynamic quantification throughout the reaction (49). A substitute to probe-based methodologies in quantitative PCR is the use of SYBR Green, a highly sensitive, double-stranded DNA-binding dye (52). This approach depends on the dye's capacity to intercalate with any double-stranded DNA generated during amplification, thereby permitting real-time detection of the PCR product (51).

One of the important advantages of SYBR Green-based assays is their flexibility; the same dye and master mix can be useful across a range of targets, eliminating the need for synthesising pathogen-specific probes, as required in probe-based systems (51). Although intercalating dyes such as SYBR Green may bind to nonspecific amplification products, the application of hot-start PCR protocols suggestively enhances assay specificity (52). This mechanism suppresses non-specific primer binding and extension during the initial stages of the reaction, thereby restraining mispriming events and ensuring that fluorescence signals reflect only the amplification of the proposed target sequence (51,52).

Among the traditional laboratory diagnostic methods of several laboratory methods, namely PCR, serology, and culture, each varies significantly depending on the viral pathogen and the specimen type (52). PCR remains the foundation for the rapid and specific detection of viral nucleic acids in CNS infections, with CSF being the most enlightening sample across a broad spectrum of neurotropic viruses (53). For instance, in the detection of herpesviruses, particularly HSV-1, HSV-2 and VZV, PCR applied to CSF yields high sensitivity, making it the diagnostic method of choice as shown in table 1. Skin vesicles or brain tissue may be used as substitute specimens in VZV and HSV infections, but CSF PCR remains most applied in clinical settings (55). For viruses such as HSV types 1 and 2 (HSV-1 & HSV-2), VZV and HHV-6, CSF remains the specimen of choice, particularly in cases presenting with encephalitis or meningitis, with few other viruses with the CNS symptoms and other useful specimens to take if CSF is not available (53,54,55).

Steiner et al. (2010) highlight the clinical utility of CSF PCR as a primary diagnostic tool, noting that its superior sensitivity and specificity provide an unparalleled diagnostic yield compared to alternative protocols during the early stages of viral infection (6). The incorporation of CSF PCR testing with imaging and serology provides a robust diagnostic framework, while other modalities such as electroencephalogram (EEG), viral culture, and biopsy are applied selectively based on clinical situation and disease progression (40,42,45,48).

Multiplex PCR

The early and precise diagnosis of viral meningoencephalitis is critical for patient management and prognosis, predominantly due to the widespread range of potential viral etiologies (56). Multiplex PCR has emerged as a powerful diagnostic tool for the rapid and simultaneous detection of multiple viral pathogens in a single reaction from CSF, refining diagnostic yield significantly and reducing both time and cost compared to singleplex traditional PCR methods (53,55,57). Liesman et al. (2018) assessed a commercial multiplex molecular panel and found it to be exceedingly effective in diagnosing common pathogens involved in infectious meningitis and encephalitis, such as HSV-1, HSV-2, VZV and enteroviruses, as well as it could effectively detect CMV and EBV, which are particularly relevant in immunocompromised populations (58). The panel established a fast turnaround time and good sensitivity, giving clinicians a timely tool for diagnosis (58).

Similarly, Shi et al. (2016) developed a novel multiplex PCR Mag-Array system that effectively detected 13 different viruses commonly related with meningoencephalitis, demonstrating high sensitivity and specificity as for example, ranging from 98.78% to 100% for HSV-1 and HSV-2, providing a promising stage for clinical laboratories targeting for comprehensive viral screening in neurological infections (59). Furthermore, the clinical efficacy of multiplex PCR is reinforced by its high diagnostic accuracy in immunocompromised patients, where extensive pathogen coverage is crucial (59).

In recent years, digital PCR (dPCR) has emerged as a transformative molecular platform for the diagnosis of infectious diseases, offering noteworthy clinical advantages over standard quantitative PCR (60). By enabling highly accurate, absolute quantification of pathogenic nucleic acids without the need for standard calibration curves, dPCR may streamline the diagnostic process (60). Furthermore, it enhanced sensitivity and high tolerance to the inhibitory substances often found in complex clinical samples make it a robust tool for facilitating rapid and reliable clinical decisions (60).

Next-generation sequencing (NGS) technology

Despite its advantages, multiplex PCR does present certain drawbacks. False negatives may happen, mainly during the early stages of infection when viral loads are low (6). Moreover, the limited panel of viruses targeted by commercial kits can be a disadvantage when dealing with rare or emerging pathogens (58). In such scenarios, next-generation sequencing (NGS) technologies can supplement PCR-based approaches (61). For instance, Guan et al. (2016) applied NGS to detect viral genomes in CSF from patients with meningoencephalitis and identified pathogens that were not covered in standard PCR panels (62). Although NGS offers comprehensive pathogen detection, it remains constrained by significant expenses and extended turnaround times (TAT) (63).

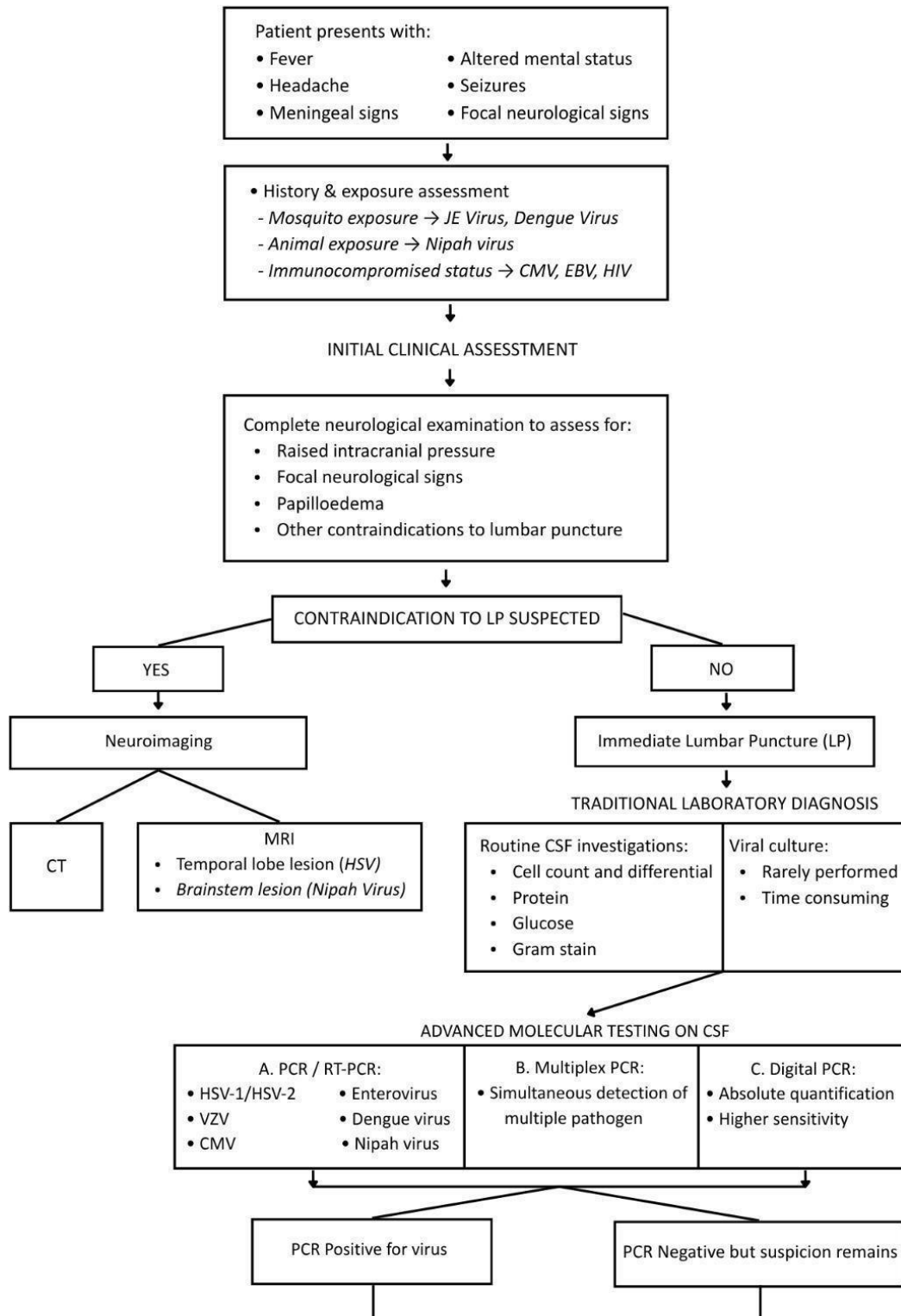
Metagenomic next-generation sequencing (mNGS) has emerged as a transformative diagnostic tool for infectious diseases like viral meningoencephalitis, demonstrating significantly higher sensitivity in identifying causative pathogens compared to routine microbiological assessments, as evidenced by a 213-patient study by Xing et al. (64). Supporting these findings, additional research has similarly established the superior diagnostic yield of mNGS compared to standard PCR and culture-based techniques (62, 63). Unlike targeted PCR methods that necessitate prior expectations about potential pathogens, mNGS allows for unbiased detection of an extensive array of pathogens, including novel or unexpected viruses, directly from clinical specimens such as CSF (62, 63). This broad-spectrum aptitude is particularly critical in immunocompromised patients or those with atypical clinical presentations, where diagnostic uncertainty is high (63-65). Yao et al. (2025) also highlighted its effectiveness in detecting EBV CNS infections, where conventional assays frequently produce false negatives due to low viral loads or mutations in primer

regions (66). Moreover, the ability of mNGS to detect co-infections or rare pathogens further strengthens its diagnostic role in complex neurological presentations (66,67). Apart from providing these enhanced diagnostic capabilities, Nguyen et al. (2020) emphasised the clinical utility of mNGS within under-resourced environments where traditional testing methods are frequently nonexistent or impractical (65).

Although mNGS delivers an unbiased diagnostic paradigm, its adoption within routine clinical workflows remains constrained not only by the previously noted financial and extended turnaround time (TAT) limitations, but additionally by the critical requirement for advanced bioinformatic interpretation expertise (64,65). The interpretation of sequencing data remains challenging due to the potential background contamination and the difficulties in distinguishing between active infection and incidental findings (62). On the other hand, targeted next-generation sequencing (tNGS) involves selective sequencing of specific genomic regions using methods such as PCR amplification or probe hybridisation, which allows for high sensitivity in pathogen detection while minimising sequencing costs by focusing only on the relevant microbial nucleic acids (68). For example, probe-based hybridisation strategies have established successful detection of herpesviruses (HSV-1, HSV-2), CMV, all serotypes of human enteroviruses, and multiple flaviviruses, including WNV and Dengue viruses (43,54,59). One benefit of tNGS is its ability to simultaneously recognise resistance genes during pathogen detection, thereby enhancing its clinical effectiveness (68).

Because of the inherent challenges of mNGS, particularly the risk of low pathogen loads and its reliance on comprehensive reference databases, mNGS frequently require confirmation via targeted approaches like PCR or tNGS (61,67). Consequently, current literature strongly advocates for a multimodal diagnostic strategy. As Xu et al. (2023) emphasise, integrating serological testing with molecular methods (PCR and NGS) maximises diagnostic accuracy and pathogen identification (40). This combinatorial approach ensures a definitive diagnosis even in the most complex or high-risk clinical scenarios (69). Figure 1 illustrates a comprehensive diagnostic algorithm from the clinical assessment combining multiple testing modalities for the evaluation of viral meningoencephalitis.

Visual Diagnostic Workflow for Viral Meningoencephalitis



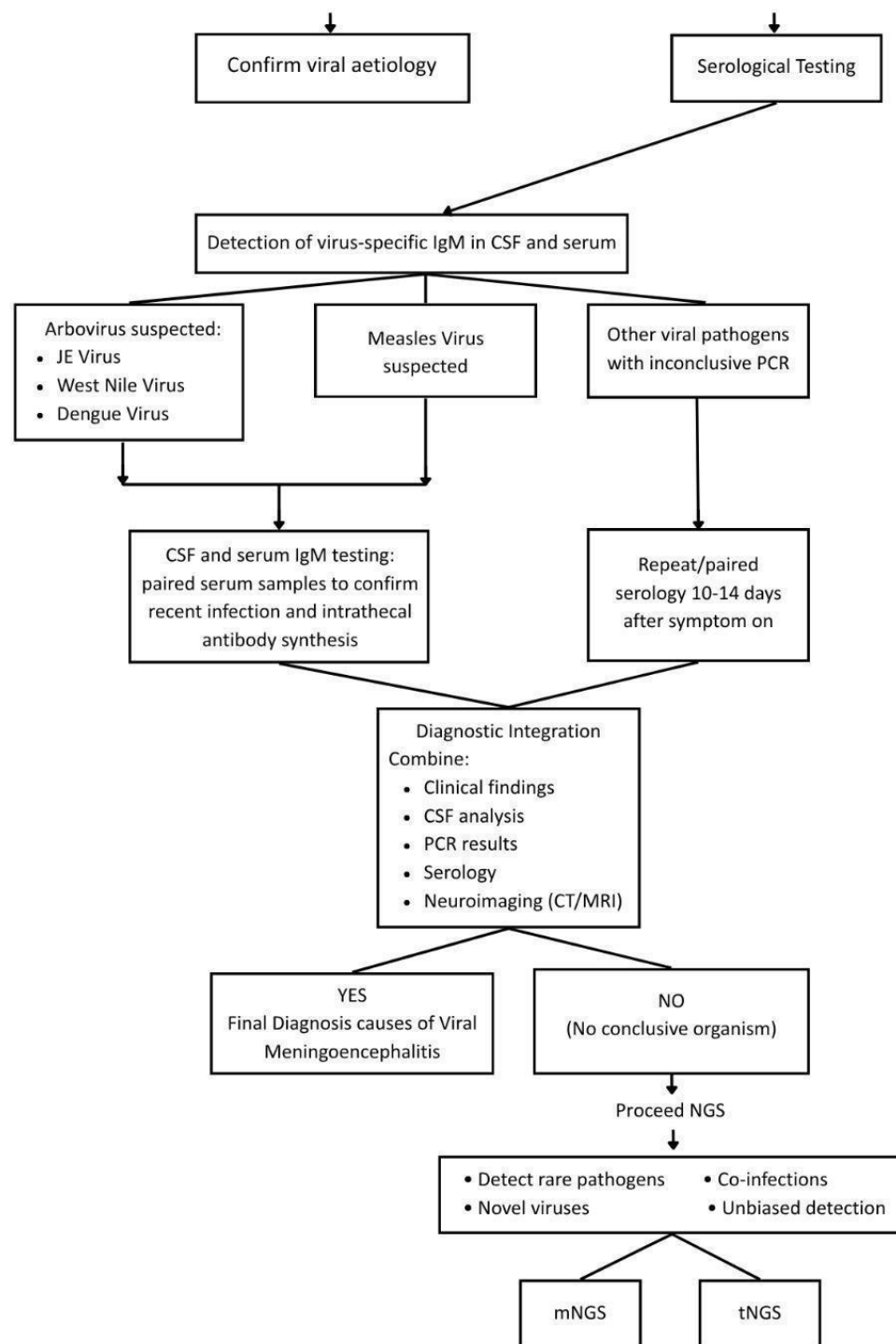


Figure 1. This is the visual diagnostic workflow for viral meningoencephalitis.

Beyond individual clinical diagnosis, NGS particularly whole-genome and mNGS also plays a pivotal role in epidemiological surveillance and outbreak management for viral meningoencephalitis. NGS allows simultaneous detection and characterisation of virtually any pathogen, including novel, rare, or unexpectedly mutated viruses that conventional, targeted molecular tests might miss (70). During an outbreak management, analysis of the high-resolution genomic data and correlating them with

epidemiological data enables the precise tracking of transmission networks, identification of infection sources and the real-time monitoring of viral evolution across diverse geographical regions (71,72). In summary, the high-resolution data provided by NGS transforms the public health response from reactive to proactive, ensuring that viral meningoencephalitis outbreaks are not only identified with precision but are also contained through swift, evidence-based interventions.

CONCLUSION

Viral meningoencephalitis remains the main neurological emergency worldwide, related with high morbidity, mortality and long-term complications. The condition, defined by inflammation of both the meninges and brain parenchyma, is caused by an extensive spectrum of viruses with epidemiological outlines predisposed by geography, host immunity and environmental factors. In Malaysia, outbreaks of JE, Nipah virus, and EV-A71 highlight the substantial public health and socioeconomic impact of emerging and re-emerging pathogens. As diagnostic technology advances, the incorporation of traditional methods, serological, molecular and genomic platforms will enhance our capacity for accurate pathogen identification, improve patient outcomes and strengthen surveillance systems. The future of viral meningoencephalitis management lies not only in rectifying diagnostic accuracy but also in building regional and global networks for data sharing, outbreak response and continuous research. Collaborative efforts between clinicians, laboratorians, epidemiologists and policymakers are essential to guarantee that lessons from past outbreaks translate into sturdier preparedness for impending threats.

In conclusion, viral meningoencephalitis demonstrates the enduring challenges of infectious diseases at the interface of neurology, microbiology, and public health. Continued investment in advanced diagnostic tools, vaccination programs, vector control approaches and international collaboration is critical. With the emergence of high-throughput technologies such as NGS, the landscape of diagnosis and management is being redesigned, offering confidence for more detailed, timely and effective interventions. Ultimately, strengthening both laboratory capacity and public health infrastructure will be pivotal in reducing the burden of viral meningoencephalitis and safeguarding neurological health globally.

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Data Availability: Not applicable.

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REFERENCES

1. Lyons JL. Viral meningitis and encephalitis. *Continuum (Minneap Minn)*. 2018;24(5):1284-1297. doi: 10.1212/CON.0000000000000650.
2. Rotbart HA. Viral meningitis. *Semin Neurol*. 2000;20(3):277-292. doi: 10.1055/s-2000-9427.
3. Walker DH. Principles of diagnosis of infectious diseases. In: McManus LM, Mitchell RN, editors. *Pathobiology of human disease*. San Diego: Elsevier; 2014. p. 222-225. doi: 10.1016/B978-0-12-386456-7.01713-5.
4. Kohil A, Jemmieh S, Smatti MK, Yassine HM. Viral meningitis: an overview. *Arch Virol*. 2021;166(2):335-345. doi: 10.1007/s00705-020-04891-1.
5. Piccirilli G, Chiereghin A, Gabrielli L, Giannella M, Squarzon D, Turello G, et al. Infectious meningitis/encephalitis: evaluation of a rapid and fully automated multiplex PCR in the microbiological diagnostic workup. *New Microbiol*. 2018;41(2):118-125.
6. Steiner I, Budka H, Chaudhuri A, Koskiniemi M, Sainio K, Salonen O, et al. Viral meningoencephalitis: a review of diagnostic methods and guidelines for management. *Eur J Neurol*. 2010;17(8):999-e57. doi: 10.1111/j.1468-1331.2010.02970.x.
7. Kennedy PGE, Chaudhuri A. Herpes simplex encephalitis. *J Neurol Neurosurg Psychiatry*. 2002;73(3):237-8. doi: 10.1136/jnnp.73.3.237.
8. Venkatesan A, Murphy OC. Viral encephalitis. *Neurol Clin*. 2018;36(4):705-724. doi: 10.1016/j.ncl.2018.07.001.
9. Said S, Kang M. Viral encephalitis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Aug 8 [cited 2026 Mar 18]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK470162/>.
10. Tuppeny M. Viral meningitis and encephalitis. *Crit Care Nurs Clin North Am*. 2013;25(3):363-380. doi: 10.1016/j.ccell.2013.04.003.
11. Mathew S, Al Khatib HA, Al Ansari K, Nader J, Nasrallah GK, Younes NN, et al. Epidemiology profile of viral meningitis infections among patients in Qatar (2015–2018). *Front Med (Lausanne)*. 2021;8:663694. doi: 10.3389/fmed.2021.663694.
12. DeBiasi RL, Tyler KL. Viral meningitis and encephalitis. *Continuum (Minneap Minn)*. 2006;12(2):58-94. doi: 10.1212/01.CON.0000290450.11098.b1.

13. Wong SC, Ooi MH, Abdullah AR, Wong SY, Krishnan S, Tio PH, et al. A decade of Japanese encephalitis surveillance in Sarawak, Malaysia: 1997-2006. *Trop Med Int Health*. 2008;13(1):52-5. doi: 10.1111/.1365-3156.2007.01967.x.
14. Chong H, Kunjapan S, Thayaparan T, Tong J, Petharunam V, Jusoh M, et al. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. *Can J Neurol Sci*. 2002;29(1):83-7. doi: 10.1017/S0317167100001785.
15. Looi LM, Chua KB. Lessons from the Nipah virus outbreak in Malaysia. *Malays J Pathol*. 2007;29(2):63-7.
16. Logan SAE, MacMahon E. Viral meningitis. *BMJ*. 2008;336(7634):36-40. doi: 10.1136/bmj.39409.673657.AE.
17. Melhuish A, McGill F. Viral meningitis and encephalitis. *Medicine*. 2021;49(11):675-80. doi: 10.1016/j.mpmed.2021.08.004.
18. Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, et al. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis*. 2010;10(12):835-44. doi: 10.1016/S1473-3099(10)70222-X.
19. Solomon T, Vaughn DW. Pathogenesis and clinical features of Japanese encephalitis and West Nile virus infections. In: Mackenzie JS, Barrett ADT, Deubel V, editors. *Japanese encephalitis and West Nile viruses*. Berlin: Springer; 2002. p. 171-94. doi: 10.1007/978-3-642-59403-8_9.
20. Mackenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med*. 2004;10(12 Suppl):S98-109. doi: 10.1038/nm1144.
21. Attar A, Khojah AM, Sakkakhini AM, Alshamrani H, Bamusa A, Alharbi Y, et al. Probable causative agents and demographic patterns of encephalitis, meningitis, and meningoencephalitis in a single tertiary care center. *Cureus*. 2024;16(9):e68707. doi: 10.7759/cureus.68707.
22. Knipe DM, Howley PM, Whelan S, editors. *Fields virology: emerging viruses*. 7th ed. Philadelphia: Wolters Kluwer; 2020. ISBN 9781975112547.
23. Jakobsen A, Skov MT, Larsen L, Trier Petersen P, Brandt C, Wiese L, et al. Herpes simplex virus 2 meningitis in adults: a prospective, nationwide, population-based cohort study. *Clin Infect Dis*. 2022;75(5):753-60. doi: 10.1093/cid/ciab1071.
24. Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT. Japanese encephalitis. *J Neurol Neurosurg Psychiatry*. 2000;68(4):405-15. doi: 10.1136/jnnp.68.4.405.
25. Li GH, Ning ZJ, Liu YM, Li XH. 2017. Neurological Manifestations of Dengue Infection. *Front Cell Infect Microbiol*. 2017;7:449.. doi: 10.3389/fcimb.2017.00449.
26. Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al. Zika Virus Associated with Microcephaly. *N Engl J Med*. 2016;374(10), 951–958. doi: 10.1056/NEJMoa1600651.
27. Cardoso MJ, Perera D, Brown BA, Cheon D, Chan HM, Chan KP, et al. Molecular epidemiology of human enterovirus 71 strains and recent outbreaks in the Asia-Pacific region: comparative analysis of the VP1 gene. *J Clin Microbiol*. 2003;41(9):4183-90.
28. Grapin M, Mirand A, Pinquier D, Basset A, Bendavid M, Bisseux M, et al. Severe and fatal neonatal infections linked to a new variant of echovirus 11, France, July 2022 to April 2023. *Euro Surveill*. 2023;28(22):2300253. doi:10.2807/1560-7917.ES.2023.28.22.2300253.
29. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science*. 2000;288(5470):1432-5. doi: 10.1126/science.288.5470.1432.

30. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet*. 1999;354(9186):1257-9. doi: 10.1016/S0140-6736(99)04299-3.
31. Wong KT, Shieh SK, Zaki JH, Tan P, Abdullah K, Lam SK. Neuropathology of Nipah virus encephalitis. *J Neuropathol Exp Neurol*. 2000;59(11):965-72.
32. Griffin DE. Measles virus. In: Knipe DM, Howley PM, editors. *Fields virology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 1042-69.
33. Shahan B, Choi EY, Nieves G. Cerebrospinal fluid analysis. *Am Fam Physician*. 2021;103(7):422-8.
34. Dien Bard J, Alby K. Point-counterpoint: meningitis/encephalitis syndromic testing in the clinical laboratory. *J Clin Microbiol*. 2018;56(4):e00018-18. doi: 10.1128/JCM.00018-18.
35. Venkatesan A, Tunkel AR, Bloch KC, Luring AS, Sejvar J, Bitnun A, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the International Encephalitis Consortium. *Clin Infect Dis*. 2013;57(8):1114-28. doi: 10.1093/cid/cit458.
36. Afifi AM, Abdullah JM, Siregar JA, Idris Z. A retrospective study on the first cerebrospinal fluid taken from external ventricular drainage insertion in meningitis patients with hydrocephalus. *Malays J Med Sci*. 2019;26(5):64-73. doi: 10.21315/mjms2019.26.5.6.
37. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2008;47(3):303-27. doi: 10.1086/589747.
38. Klein RS. Viral meningitis. In: *MSD Manual Professional Version* [Internet]. Rahway (NJ): Merck & Co., Inc.; 2024 Nov [modified 2025 Feb; cited 2026 May 14]. Available from: <https://www.msdmanuals.com/professional/neurologic-disorders/meningitis/viral-meningitis>.
39. Jerome KR, editor. *Lennette's Laboratory Diagnosis of Viral Infections*. 4th ed. Boca Raton, (FL): CRC Press;2016. ISBN: 9781420084962. doi: 10.3109/9781420084962.
40. Xu H, Chen P, Guo S, Shen X, Lu Y. Progress in etiological diagnosis of viral meningitis. *Front Neurol*. 2023;14:1193834. doi: 10.3389/fneur.2023.1193834.
41. Piantadosi A, Kanjilal S. Diagnostic approach for arboviral infections in the United States. *J Clin Microbiol*. 2020;58(12):e01926-19. doi: 10.1128/JCM.01926-19.
42. Gomes HR. Cerebrospinal fluid analysis: current diagnostic methods in central nervous system infectious diseases. *Arq Neuropsiquiatr*. 2022;80(5 Suppl 1):290-5. doi: 10.1590/0004-282X-ANP-2022-S114.
43. Garber B, Glauser J. Viral meningitis and encephalitis update. *Curr Emerg Hosp Med Rep*. 2024;12:95-102. doi: 10.1007/s40138-024-00294-7.
44. Schibler M, Eperon G, Kenfak A, Lascano A, Vargas MI, Stahl JP. Diagnostic tools to tackle infectious causes of encephalitis and meningoencephalitis in immunocompetent adults in Europe. *Clin Microbiol Infect*. 2019;25(4):408-14. doi: 10.1016/j.cmi.2018.12.035.
45. Perillo T, Capasso R, Pinto A. Neuroimaging of the most common meningitis and encephalitis of adults: a narrative review. *Diagnostics*. 2024;14(11):1064. doi: 10.3390/diagnostics14111064.
46. Granerod J, Davies NWS, Mukonoweshuro W, Mehta A, Das K, Lim M, et al. Neuroimaging in encephalitis: analysis of imaging findings and interobserver agreement. *Clin Radiol*. 2016;71(10):1050-8. doi: 10.1016/j.crad.2016.03.015.
47. DeBiasi RL, Tyler KL. Polymerase chain reaction in the diagnosis and management of central nervous system infections. *Arch Neurol*. 1999;56(10):1215-9. doi: 10.1001/archneur.56.10.1215.
48. Cinque P, Bossolasco S, Lundkvist Å. Molecular analysis of cerebrospinal fluid in viral diseases of the central nervous system. *J Clin Virol*. 2003;26(1):1-28. doi: 10.1016/S1386-6532(02)00173-7.

49. DeBiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. *Clin Microbiol Rev.* 2004;17(4):903-25. doi: 10.1128/CMR.17.4.903-925.2004.
50. Syed Hussin SAD, Chua AL, Al-Talib H, Sekaran SD, Wang SM. An overview of laboratory diagnosis of central nervous system viral infections. *J Pure Appl Microbiol.* 2022;16(4): 2225-2245. doi: 10.22207/JPAM.16.4.34.
51. Kricka LJ. Stains, labels and detection strategies for nucleic acids assays. *Ann Clin Biochem.* 2002;39(2):114-29. doi: 10.1258/0004563021901865.
52. Lekanne Deprez RH, Fijnvandraat AC, Ruijter JM, Moorman AFM. Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR Green I depends on cDNA synthesis conditions. *Anal Biochem.* 2002;307(1):63-9. doi: 10.1016/S0003-2697(02)00021-0.
53. Wreghitt T, Kudesia G. *Clinical and diagnostic virology.* 2nd ed. Cambridge: Cambridge University Press; 2024. p. 334.
54. Aberle SW, Puchhammer-Stöckl E. Diagnosis of herpesvirus infections of the central nervous system. *J Clin Virol.* 2002;25(Suppl 1):79-85. doi: 10.1016/S1386-6532(02)00037-9.
55. Korimbocus J, Scaramozzo N, Lacroix B, Crance JM, Garin D, Vernet G. DNA probe array for the simultaneous identification of herpesviruses, enteroviruses, and flaviviruses. *J Clin Microbiol.* 2005;43(8):3779-87. doi: 10.1128/JCM.43.8.3779-3787.2005.
56. Read SJ, Kurtz JB. Laboratory diagnosis of common viral infections of the central nervous system by using a single multiplex PCR screening assay. *J Clin Microbiol.* 1999;37(5):1352-5. doi: 10.1128/JCM.37.5.1352-1355.1999.
57. Syed Hussin SAD, Chen XW, Al-Talib H, Chua AL, Azimi Z, Wang SM. Diagnostic accuracy of multiplex real-time PCR for detecting viruses associated with encephalitis: a systematic review and meta-analysis protocol. *PLoS One.* 2025;20(3):e0318805. doi: 10.1371/journal.pone.0318805.
58. Liesman RM, Strasburg AP, Heitman AK, Theel ES, Patel R, Binnicker MJ. Evaluation of a commercial multiplex molecular panel for diagnosis of infectious meningitis and encephalitis. *J Clin Microbiol.* 2018;56(4):e01927-17. doi: 10.1128/JCM.01927-17.
59. Shi X, Wu R, Shi M, Zhou L, Wu M, Yang Y, et al. Simultaneous detection of 13 viruses involved in meningoencephalitis using a newly developed multiplex PCR Mag-Array system. *Int J Infect Dis.* 2016;49:80-6. doi: 10.1016/j.ijid.2016.05.023.
60. Sancha Dominguez L, Cotos Suárez A, Sánchez Ledesma M, Muñoz Bellido JL. Present and future applications of digital PCR in infectious diseases diagnosis. *Diagnostics.* 2024;14(9):931. doi: 10.3390/diagnostics14090931.
61. Kanaujia R, Biswal M, Angrup A, Ray P. Diagnostic accuracy of the metagenomic next-generation sequencing (mNGS) for detection of bacterial meningoencephalitis: a systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis.* 2022;41(6):881-91. doi: 10.1007/s10096-022-04445-0.
62. Guan H, Shen A, Lv X, Yang X, Ren H, Zhao Y, et al. Detection of virus in CSF from the cases with meningoencephalitis by next-generation sequencing. *J Neurovirol.* 2016;22(2):240-5. doi: 10.1007/s13365-015-0390-7.
63. Carbo EC, Blankespoor I, Goeman JJ, Kroes ACM, Claas ECJ, de Vries JJC. Viral metagenomic sequencing in the diagnosis of meningoencephalitis: a review of technical advances and diagnostic yield. *Expert Rev Mol Diagn.* 2021;21(11):1139-46. doi: 10.1080/14737159.2021.1985467.
64. Xing XW, Zhang JT, Ma YB, He MW, Yao GE, Wang W, et al. Metagenomic next-generation sequencing for diagnosis of infectious encephalitis and meningitis: a large, prospective case series of 213 patients. *Front Cell Infect Microbiol.* 2020;10:88. doi: 10.3389/fcimb.2020.00088.

65. Hong NTT, Anh NT, Mai NTH, Nghia HDT, Nhu LNT, Thanh TT, et al. Performance of metagenomic next-generation sequencing for the diagnosis of viral meningoencephalitis in a resource-limited setting. *Open Forum Infect Dis.* 2020;7(3):ofaa046. doi: 10.1093/ofid/ofaa046.
66. Yao X, Huang Y, Yuan L, Han D, Zhang X. Clinical performance of metagenomic next-generation sequencing in the diagnosis of Epstein–Barr virus central nervous system infections. *Eur J Clin Microbiol Infect Dis.* 2025;44(11):2623-2634.doi: 10.1007/s10096-025-05227-0.
67. Zhang JT, Xing XW, Ma YB, He MW, Qi XK, Wang W, et al. Metagenomic next-generation sequencing for diagnosis of infectious encephalitis and meningitis: a large, prospective case series of 213 patients. *Front Cell Infect Microbiol.* 2020;10:88. doi: 10.3389/fcimb.2020.00088.
68. Perlejewski K, Bukowska-Ośko I, Rydzanicz M, Pawełczyk A, Caraballo Cortès K, Osuch S, et al. Next-generation sequencing in the diagnosis of viral encephalitis: sensitivity and clinical limitations. *Sci Rep.* 2020;10(1):16173. doi: 10.1038/s41598-020-73156-3.
69. Cunha BA. The clinical and laboratory diagnosis of acute meningitis and acute encephalitis. *Expert Opin Med Diagn.* 2013;7(4): 343-64 . doi: 10.1517/17530059.2013.804508.
70. Aulicino PC, Kimata JT. Beyond surveillance: leveraging the potential of next generation sequencing in clinical virology. *Front Trop Dis.* 2024;5:1512606. doi: 10.3389/fitd.2024.1512606.
71. Maljkovic Berry I, Melendrez MC, Bishop-Lilly KA, Rutvisuttinunt W, Pollett S, Talundzic E, Morton L, et al. Next generation sequencing and bioinformatics methodologies for infectious disease research and public health: approaches, applications, and considerations for development of laboratory capacity. *J Infect Dis.* 2020;221(Suppl 3):S292-S307. doi: 10.1093/infdis/jiz286.
72. Alsharksi AN, Sirekbasan S, Gürkök-Tan T, Mustapha A. From tradition to innovation: diverse molecular techniques in the fight against infectious diseases. *Diagnostics (Basel).* 2024;14(24):2876. doi: 10.3390/diagnostics14242876.