

Laboratory Diagnosis of Central Nervous System Infection

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Abstract Central nervous system (CNS) infections are potentially life threatening if not diagnosed and treated early. The initial clinical presentations of many CNS infections are non-specific, making a definitive etiologic diagnosis challenging. Nucleic acid in vitro amplification-based molecular methods are increasingly being applied for routine microbial detection. These methods are a vast improvement over conventional techniques with the advantage of rapid turnaround and higher sensitivity and specificity. Additionally, molecular methods performed on cerebrospinal fluid samples are considered the new gold standard for diagnosis of CNS infection caused by pathogens, which are otherwise difficult to detect. Commercial diagnostic platforms offer various monoplex and multiplex PCR assays for convenient testing of targets that cause similar clinical illness. Pan-omic molecular platforms possess potential for use in this area. Although molecular methods are predicted to be widely used in diagnosing and monitoring CNS infections, results generated by these methods need to be carefully interpreted in combination with clinical findings. This review summarizes the currently available armamentarium of molecular assays for diagnosis of

central nervous system infections, their application, and future approaches.

Keywords Central nervous system infections · Meningitis · Encephalitis · Laboratory diagnosis · Microscopic morphology · Rapid antigen testing · Culture · Serology · Molecular methods · Pan-omic techniques

Introduction

The central nervous system (CNS) has unique anatomic and immunologic characteristics that play an important role in the pathogenesis and detection of infection. CNS is protected by the blood–brain barrier (BBB) but is still highly vulnerable to microbial invasion by extension from a contiguous focus; hematogenous dissemination; or less commonly, intraneural passage of organisms [1]. Various environmental or commensal bacteria, viruses, fungi, protozoa, or parasites can migrate into CNS causing a variety of infections [2•]. Clinical manifestation of CNS infection can consist of fever, headache, vomiting, photophobia, stiff neck, and focal neurological presentations.

CNS infections are classified according to their anatomic localization (Fig. 1) [2•, 3, 45•]. Infection of the meninges, brain, and spinal cord results in meningitis, encephalitis, brain abscess, and myelitis, respectively. Infection may be limited to a single anatomic compartment or may involve multiple sites (e.g., meningoencephalitis and encephalomyelitis). Based on the duration, infection can be classified as acute, sub-acute, chronic, or recurrent. Meningitis is characterized by the onset of fever, headache, neck stiffness, and photophobia over a period of hours to days. Encephalitis is characterized by brain parenchymal inflammation, and the clinical hallmark is alteration in mental status, ranging from lethargy to coma [6].

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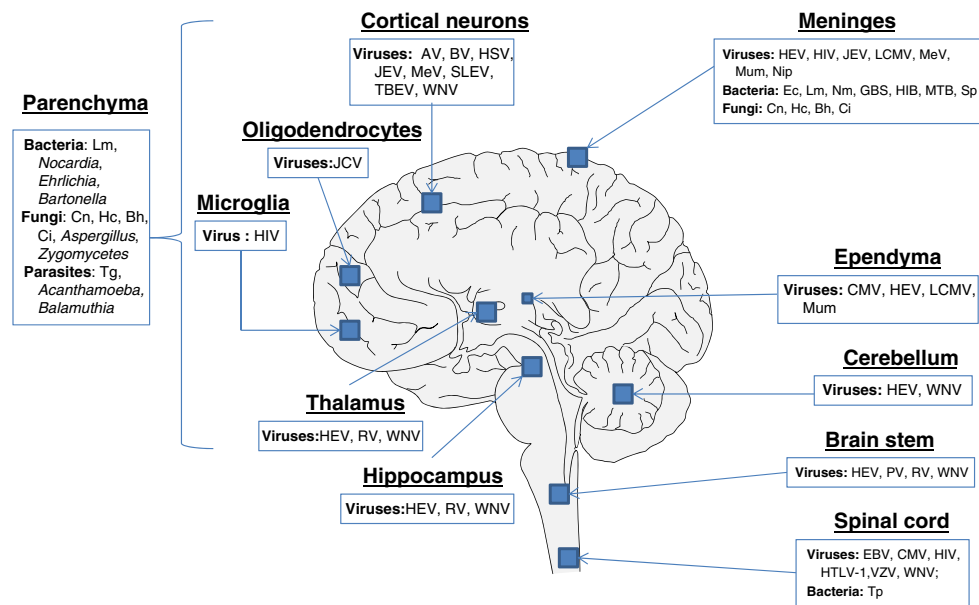


Fig. 1 A diagram of nervous system anatomy and the specific pathogens associated with each structure. Modified with permission from Swanson and McGavern [5•]. AVs alphaviruses, BVs bunyaviruses, CMV cytomegalovirus, HEVs human enteroviruses, HIV human immunodeficiency virus, HSV herpes simplex virus, JCV John Cunningham virus, JEV Japanese encephalitis virus, LCMV lymphocytic choriomeningitis virus, MeV measles virus, Mum mumps virus, Nip Nipah virus, PV poliovirus, RV rabies virus, SLEV St. Louis

encephalitis virus, TBEV tick-borne encephalitis virus, WNV West Nile virus, Lm *Listeria monocytogenes*, Nm *Neisseria meningitidis*, Ec *Escherichia coli*, Cn *Cryptococcus neoformans*, Hc *Histoplasma capsulatum*, Bh *Blastocystis hominis*, Ci *Coccidioides immitis*, Td *Toxoplasma gondii*, GBS Guillain-Barré syndrome, HIB *Haemophilus influenzae* type b, MTB *Mycobacterium tuberculosis*, Sp *Streptococcus pneumoniae*, Tp *Treponema pallidum*

Myelitis is characterized by the inflammation of the spinal cord with symptoms including fever, headache, and paraparesis or paralysis. Based on the duration, infection can be classified as acute, sub-acute, chronic, or recurrent.

The most rapidly fatal infection of the CNS is acute bacterial meningitis, with an annual incidence of 3 to 5 cases per 100,000 persons in the USA and an associated mortality rate of 6 to 26 % [2••]. Annually, approximately 4000 cases of acute bacterial meningitis occur in the USA with 500 deaths. The leading causes of bacterial meningitis across all age groups include *Streptococcus pneumoniae*, group B *Streptococcus*, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Listeria monocytogenes*.

CNS infections caused by viruses are more common and mostly mild and self limited. These can clinically manifest as meningitis and/or encephalitis [7]. The incidence of viral CNS infections can vary by geographic region and season. Non-polio enteroviruses account for majority of meningitis/encephalitis cases that typically peak between late spring to fall [8•]. The more serious CNS infections due to herpes simplex viruses (HSVs) are associated with sporadic encephalitis and meningitis with severe sequelae if not treated promptly [9].

Rapid and accurate detection and identification of microbial pathogens is essential in directing timely clinical intervention. Conventional methods used in clinical microbiology laboratories include direct microscopic examination, culture

techniques, antigen, and antibody detection assays (Table 1). These methods although currently applied have several important limitations—using the example of enteroviruses, the most common cause of meningitis [10••, 11]. Direct microscopic examination of cerebrospinal fluid (CSF) has limited sensitivity and specificity. The sensitivity of culture for enteroviruses is between 65 and 75 % with a mean retrieval time of 3.7 to 8.2 days [12]. Furthermore, some serotypes of enteroviruses, especially *Coxsackievirus A* strains, are known to grow poorly or are non-cultivable [13]. Enteroviruses lack a common antigen among various serotypes making a universal antigen or antibody detection impossible. Similar issues occur around the diagnosis of CNS HSV infections by conventional methods—culture sensitivity from CSF is extremely poor. Presence of HSV IgG antibodies in CSF can be used in diagnosis; however, production is delayed until day 10 or 12 after infection and is therefore not ideal for early diagnosis [14].

To overcome the several limitations of conventional diagnostic techniques, molecular methods, dominantly PCR-based amplification, have gradually become mainstay tools in detection and identification of microbial pathogens in CSF (Table 1) [2••, 15]. When compared to conventional methods, molecular methods show greater detection rates; one study reported that 16S ribosomal ribonucleic acid (rRNA) PCR-based assays were able to accurately detect the causative organism in 65 % of banked CSF samples, compared to 35 % when using culture and microscopy [16]. In another report,

Table 1 Laboratory methods used for diagnosis of CNS infections

Test	Ease of performance	Turnaround time	Result interpretation	Advantages	Disadvantages	Selected references
Microscopic examination	Could be performed in routine clinical lab	0.5–1 h	Direct if correlated with symptoms	Rapid	Poor sensitivity and specificity; special skills are needed for interpretation	[23–26]
Rapid antigen	Could be performed in routine clinical lab	15–30 min	Direct if correlated with symptoms	Rapid	False positive results	[31, 33, 34, 37, 38]
Culture	Could be performed in sophisticated clinical lab	2–14 days	Definite	For phenotypic drug susceptibility testing	Time consuming and poor sensitivity; limited microorganisms are culturable	[25, 27, 29]
Serology	Could be performed in routine clinical lab	2–8 h	Indirect	Automation	Results are generally retrospective; cross-reactions; immunosuppressed host may be unable to mount a response	[45–47]
Molecular diagnostic	Could be performed in routine clinical lab with trend in point of care	2–8 h	Direct without knowing microbial viability	High sensitivity and specificity	Not the test of cure; clinical relevance need to be determined	[8, 51, 59, 60, 79, 80]

improved diagnostic yield based on molecular methods was used to optimize antibiotic treatment of patients with infectious meningitis when conventional methods provided a negative result [17]. Currently, molecular methods performed on CSF samples are considered a “platinum” standard, in contrast of the culture gold standard, in diagnosis of CNS infections caused by viruses which are difficult to detect and identify [18–20].

Since we provided a review on the topic in 2002 [21•], the diagnostic landscape in CNS infections has changed dramatically. PCR-based molecular methods made their way into clinical microbiology laboratory, providing tools for rapid and accurate diagnosis. In parallel, several commercial molecular assays as summarized in Table 2 have been cleared by the Food and Drug Administration (FDA) for detection of target microbial pathogens in CSF.

Despite the advances in molecular techniques, several challenges remain. Using a combination of conventional and molecular diagnostic methods, Glaser et al. showed that in approximately 62 % of patients with clinical encephalitis, an etiologic organism could not be identified [22]. The focus is now shifting towards development of advanced techniques beyond nucleic acid-based detection. In this review, we give an update on the existing conventional and molecular platforms for the diagnosis of CNS infections. We also provide a preview on the potential clinical application of future technologies including pan-omic assays. The emphasis is given to optimal test selection based on the clinical scenario.

Conventional Microbiology Methods

Microscopic Examination

A positive CSF Gram stain is highly suggestive of bacterial meningitis [23, 24]. The reported sensitivity of the Gram stain for diagnosis of bacterial meningitis is 60~80 % in patients who have not received antimicrobial treatment and 40~60 % among those on antibacterial treatment [25]. In one study, Gram stain detected as many as 90 % *S. pneumoniae* and 50 % *L. monocytogenes* in CSF collected from patients with bacterial meningitis confirmed by PCR [26]. Two organisms which are often diagnosed by microscopy are *Mycobacterium tuberculosis* by acid-fast bacillus (AFB) staining and *Cryptococcus neoformans* by India ink or Gram stain. While these methods maintain satisfactory specificities, the sensitivities are relatively poor; thereby, a culture is usually performed in parallel.

Culture

Culture of brain tissue can provide definitive diagnosis of CNS infections; however, obtaining biopsies is highly

Table 2 Commercially available FDA-cleared or FDA-approved assays for pathogen detection in the CNS

Manufacturer	Assay name	Organism	Technology	Specimen type	Comments	References
Cepheid, Sunnyvale, CA	Xpert EV Assay	Enteroviruses	Real-time PCR	CSF	Target is the 5' untranslated region; fully integrated and random access; 2-h 30' TAT	[51]
bioMérieux, Durham, NC	NucliSENS EasyQ Enterovirus assay	Enteroviruses	NASBA	CSF	Target is the 5' untranslated region; separate nucleic acid and amplification/detection steps, automated; 5-h TAT	[60]
Focus Diagnostics, Cypress, CA	Simplexa HSV-1&2 Direct	HSV-1, HSV-2	Real-time PCR	CSF; genital lesions	Target is the DNA polymerase; semi-automated; no extraction; 1-h TAT	[59]
BioFire Diagnostics, Salt Lake City, UT	FilmArray Meningitis/Encephalitis panel	EcK1, Hi, Lm, Nm, Sa, Sp, CMV, enterovirus, HSV-1, HSV-2, HHV-6	Multiplex PCR followed by solid array	CSF	Fully integrated and random access; approximately 1-h TAT	[79, 80]

NASBA nucleic acid sequence-based amplification, HSV herpes simplex virus, CMV cytomegalovirus, HHV-6 human herpesvirus 6, CSF cerebrospinal fluid, TAT test turnaround time, EcK1 *E. coli* K1, Hi *Haemophilus influenzae*, Lm *Listeria monocytogenes*, Nm *Neisseria meningitidis*, Sa *Streptococcus agalactiae*, Sp *Streptococcus pneumoniae*

invasive and often avoided unless deemed necessary by a clinician. CSF sampling is most commonly performed to determine etiology of suspected CNS infection [25, 27]. CSF viral, bacterial (including mycobacterial), and fungal cultures remain the mainstay in the diagnosis of infectious meningitis. However, the yield of CSF cultures in suspected cases is low [28]. Another disadvantage of CSF bacterial culture is that it requires up to 72 h for final identification. A recent study reported that CSF mycobacterial culture had a sensitivity of 22 % and a specificity of 100 % in diagnosis of tuberculosis meningitis [29]. For viruses, the application of monoclonal antibodies in shell vial culture has increased the speed and specificity. However, due to the long time and low sensitivity, CSF viral culture is often unable to provide the timely diagnosis required for optimum patient management [11, 30].

Rapid Antigen Detection

Among the antigen assays for CNS infections, *Cryptococcal* antigen is the most widely used. The test relies on detection of *Cryptococcus* capsular polysaccharide antigens in CSF by enzyme immunoassay [31]. In a single report that examined patients >35 years of age with CNS cryptococcosis, an overall sensitivity and specificity of 93~100 and 93~98 %, respectively, were reported [32]. *Cryptococcus* is a neurotropic fungus; polysaccharide serum antigen titers in conjunction with host immune status are often used as a diagnostic aid to determine need for lumbar puncture to evaluate patient for CNS involvement. The baseline peak titer of polysaccharide antigen in serum or CSF has demonstrated important prognostic significance [33] with higher titer (peak titer >1:1024) associated with antifungal therapy failure [34].

Detection of galactomannan (GM) antigen and (1,3)- β -D-glucan (BDG) in CSF can aid in the diagnosis of CNS aspergillosis or other invasive fungal infection such as fusariosis [10•, 35]. Elevated BDG in serum as well as CSF is associated with fungal infections; measuring the levels of BDG might be a useful biomarker in the evaluation of fungal CNS disease [36]. It was recently reported that patients receiving effective antifungal therapy showed reduction in CSF BDG concentration (<31 pg/ml), and for this reason, BDG titers in CSF can be as a useful biomarker in monitoring response to treatment [37].

For acute bacterial meningitis, a rapid antigen assay is available to detect pneumococcal capsular antigen [38]. Recently, several reports revealed potential application of detection of *M. tuberculosis*-specific antigens in CSF as rapid diagnosis of tuberculosis meningitis [39, 40]. The level of *M. tuberculosis* early secreted antigenic target 6 (ESAT-6) was associated with clinical severity and may be used for the prognosis of tuberculosis meningitis [41, 42].

Serology

Definitive serological diagnosis of CNS infections is established by detecting IgM antibodies or demonstrating a at least a fourfold increase in neutralizing antibody titers between acute- and convalescent-phase CSF [43, 44]. In general, due to delay in antibody response after symptom onset, a negative antibody test cannot be used to rule out infections and retesting may be required. In addition, in certain selected populations such as immunocompromised individuals, the tests may not offer optimum sensitivity. In most circumstances, nucleic acid amplification tests have surpassed antibody-based detection as the test of choice [11]. For certain infections, these assays still have a valuable role. CSF IgM is the most widely used test for West Nile virus (WNV) infections; antibody may appear as early as 3 days and persist for up to 3 months. However, its accuracy is complicated by high cross-reactivity with other clinically relevant flaviviruses and related vaccines [45]. Antibodies against recombinant WNV E proteins have been proposed as a potential solution to make this important distinction in areas where cross-reacting viruses co-circulate or in individuals who have been immunized [45].

Other highly important serological assays for CNS infections are tests used for screening and diagnosis of neurosyphilis. Neurosyphilis can be confirmed by a positive CSF venereal disease research laboratory (VDRL) test [46]. Detection of antibodies to varicella zoster virus (VZV) IgG and/or DNA in CSF remain as the most commonly used methods to establish a diagnosis of VZV-related CNS disease [47].

Molecular Methods in Diagnosis of CNS Infections

Because of higher sensitivity and specificity, nucleic acid in vitro amplification-based molecular techniques are now widely implemented across clinical laboratories in the USA. Molecular methods have dramatically improved the ability to diagnose CNS infections in a reasonable and effective time frame. Several PCR-derived techniques have collectively expanded the flexibility and rigor of currently available laboratory diagnostic methods [48, 49].

Reverse transcriptase (RT)-PCR was developed to amplify RNA targets; its application has played an important role in diagnosing RNA-virus infections and in some cases monitoring response to therapy. Timely access to enterovirus RT-PCR results have been shown to facilitate shorter hospital stays, reduce unnecessary antibiotic use, and lessens ancillary laboratory testing [50–52]. Broad-range rRNA PCR techniques, which use single pair of primers targeting conserved regions of genes, are successfully used for rapid detection and identification of bacterial pathogens and herpesviruses in the CSF [16, 53, 54]. Isothermal amplification-based techniques including loop-mediated isothermal amplification (LAMP) has

been successfully developed to provide point-of-care diagnosis within minutes to hours [55]. Table 2 provides an overview and comparison of commercial molecular in vitro diagnostic devices (IVDs) that have been cleared by the US FDA for detection and identification of microbial pathogens in CSF. Details and updates concerning these devices are available from the FDA website (<http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial>). In the following section, we will discuss three different types of molecular testing with focuses on CNS infection diagnosis.

Monoplex Assays

A conventional molecular procedure includes the following three separate steps: sample extraction, target nucleic acid amplification, and amplicon detection. One of the first molecular assays used successfully for CNS infection diagnosis was for detection of HSV in CSF [56]. PCR quickly became the test of choice when studies demonstrated that CSF PCR was equivalent to culture of brain tissue for diagnosis of HSV encephalitis and meningitis [15]. Since then, numerous PCR-based methods for herpesvirus and enterovirus have become available with superior sensitivity compared to viral culture [57, 58].

Real-time PCR with simultaneous nucleic acid amplification and amplicon detection further accelerated the transition to molecular testing in clinical laboratories. Unlike conventional PCR, the real-time system is a “closed” system and therefore overcomes the important concern of carryover contamination. At the time of manuscript preparation, three molecular assays to detect HSV [59] and enteroviruses [51, 60] in CSF have been approved by FDA as shown in Table 2 [61••].

Real-time PCR-based methods are the main format to detect Zika virus, which was first reported in Uganda in 1947 and is now a worldwide concern after the virus spread widely in Brazil and Central America [62]. Faye et al. developed a one-step RT-PCR assay to detect Zika virus in human serum with limit of detection of 7.7 pfu/reaction [63, 64]. In addition to plasma, Zika virus RNA can be detected in the urine and plasma within first 2 weeks after symptom onset [65]. In March 2016, the FDA approved a triplex-PCR assay under emergency use authorization for the simultaneous detection of Zika, Chikungunya, and dengue viruses in serum, urine, CSF, and amniotic fluid. The RT-PCR assay uses dual-labeled hydrolysis probes with a LOD of 1.54×10 GCE/ml [4] of Zika virus in serum (<http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491592.pdf>).

Introduction of real-time PCR-based diagnostic assays has had a substantial impact on early and effective diagnosis of certain bacterial infections [66, 67]. Isothermal amplification-based molecular assays have excellent performance characteristics and

have the distinct advantage of not requiring any specialized equipment. These assays are therefore ideal for use as on or near point-of-care testing. Using this technology, LAMP-based methods have been used to detect *Neisseria meningitidis*, *S. pneumoniae*, *H. influenzae* type b, *M. tuberculosis*, and Japanese encephalitis virus (JEV) in the CSF [68–71]. The Xpert MTB/RIF assay has revolutionized the landscape of global tuberculosis control by providing an integrated and automated system that enables rapid clinical decision making in a POC or near-care context [67]. Several studies have applied the Xpert MTB/RIF to evaluate detection of *M. tuberculosis* in CSF from cases of TB meningitis [72, 73, 74]. In a meta-analysis of 13 studies, the pooled sensitivity of Xpert assay was 80.5 % (95 % CI 59.0–92.2) against culture and 62.8 % (95 % CI 47.7–75.8) against composite standard. Using a large volume of sample (at least 8–10 ml) is required for testing CSF, and centrifugation can lead to modest improvement in yield [75]. Despite the lack of standardization for sample processing, WHO has endorsed testing CSF with the automated Xpert MTB/RIF assay as a first-line test over conventional microscopy in patients with suspected TB meningitis.

Multiplex Assays

Relative simplicity and high-throughput detection make multiplex molecular assays an attractive option for screening and detection of a panel of microbial targets [76]. Several multiplex PCR assays have been developed to identify bacterial pathogens in CSF, targeting the most common causes of meningitis, *S. pneumoniae*, *N. meningitidis*, *H. influenzae* [26, 48, 76, 77], *L. monocytogenes* [26, 77], *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli* [26], and *Mycoplasma pneumoniae* [26]. A multiplex PCR followed by Luminex suspension array can simultaneously detect eight bacterial and viral pathogens in CSF including *N. meningitidis*, *S. pneumoniae*, *E. coli*, *S. aureus*, *L. monocytogenes*, *S. agalactiae*, HSV-1/2, and VZV [78].

Considering the breadth of pathogens implicated in CNS infection, application of comprehensive molecular panels with multiple bacterial and viral targets have improved the diagnostic efficiency. The BioFire FilmArray Meningitis/Encephalitis panel is currently the only FDA-cleared multiplex assay for the detection of six bacterial (*E. coli* K1, *H. influenzae*, *L. monocytogenes*, *N. meningitidis*, *S. agalactiae*, and *S. pneumoniae*), seven viral (cytomegalovirus, enterovirus, HSV-1, HSV-2, human herpesvirus 6 (HHV-6), human parechovirus, and VZV), and single fungal (*C. neoformans/gattii*) target in CSF (Table 2). The integrated FilmArray system has a turnaround time of about an hour, with only 2 min of hands-on time. At the time of the manuscript preparation, two studies have reported on the performance of this assay [79, 80]. Using 48 samples from Gram stain-negative CSF samples from suspected cases of meningitis, Wootton et al. showed that this system detected more viral

pathogens especially EBV. Four cases of WNV and single case of *Histoplasma* were not detected by this assay. Among HIV-infected patients in Uganda, the test performance demonstrated superior sensitivity and specificity for detection of *Cryptococcus* [79, 80]. Although the FilmArray Meningitis/Encephalitis panel offers a promising platform for rapid diagnosis of CNS infections, further clinical studies are needed to determine its performance for various targets and among other high-risk populations.

Co-infections are frequently encountered among immunocompromised patients and present a difficult diagnostic challenge for clinicians. Multiplex design enables simultaneous detection and identification of multiple targets on the same sample. Rajasingham et al. [81] used a panel of monoplex and multiplex molecular assays to conduct a prospective cohort study in Uganda to comprehensively evaluate the etiology of meningitis among HIV-infected adults. Among the 314 HIV-infected patients with suspected meningitis, EBV co-infection was detected with *Cryptococcus*, *M. tuberculosis*, or other viral pathogens [81]. The clinical significance of EBV in CSF in these settings is not completely understood, although a single study associated high EBV viral load as a marker of poor outcome in individuals with bacterial meningitis and EBV co-infection/reactivation [82].

Pan-Omic Molecular Assays

Technological improvements in metagenomic deep sequencing have led to its potential application for clinical diagnosis of infections [83, 8485•]. Several reports have demonstrated its utility into solving diagnostic dilemmas that challenge the limits of traditional laboratory testing [83, 86, 8785•,]. Due to sterile status and protection by BBB, CSF and brain biopsies are ideal to further explore the application of this technology for pathogen detection and discovery. As demonstrated in a highly challenging clinical situation, metagenomics was successfully used to establish a timely diagnosis of neuroleptospirosis in a 14-year-old boy with severe combined immunodeficiency, who suffered from recurrent bouts of fever, headache, and coma [85•]. Similarly, high-throughput RNA sequencing performed on brain biopsy from an 18-month-old boy with encephalopathy was able to identify a new astrovirus as the cause [83]. Despite the enormously attractive potential of metagenomics for infectious disease diagnosis, there are many technological and practical concerns that need to be addressed before this form of diagnostic testing can become mainstream and part of the clinical standard of care.

Other promising advances have occurred in transcriptomics, proteomics, and metabolomics. Host and microbial microRNA (miRNA) profiles have been used for various inflammatory and infectious diseases [88]. Two miRNAs, miR-155 and miRNA-29b, were reported as potential biomarkers

Table 3 Application of molecular methods in detecting common pathogens causing CNS infections

Organisms	Clinical diseases/disorders	Molecular test applicability ^a	Comments
Viruses			
Adenoviruses	Meningoencephalitis	B	Serotype 7 is the common cause of CNS infection. Culture is method of choice. Commercial molecular device is available
Arboviruses	Meningoencephalitis	A-B	Arboviruses cause CNS infections including EEE, LAC, SLE, WEE, WNV, VEE, JE, POW, and RVF. Serology is the test of choice
CMV	Encephalitis, myelitis	A	Molecular detection is the test of choice. Commercial molecular device is available. Direct detection of resistance-related mutations has been reported
Enteroviruses	Meningitis	A	CSF PCR is the test of choice. Stool or throat swab PCR or culture suggestive but not diagnostic of CNS involvement. The 5'-UTR PCR detects most members of enteroviruses including EV-D68 but does not provide genotype specific information
Epstein-Barr virus	Primary CNS lymphoma, myelitis	A-B	Acute infection, serology. Reactivation disease or primary CNS lymphoma, positive CSF PCR may indicate secondary viral reactivation
HHV-6 and HHV-7	Encephalitis	A	CSF PCR. Commercial molecular device is available for HHV-6
HSV-1/HSV-2	Encephalitis, meningitis (Mollaret's meningitis)	A	CSF PCR gold standard for diagnosis. Several commercial molecular devices are available
HTLV-1/HTLV-2	Myelitis	B	Serology is the test of choice. Molecular method may enhance sensitivity
Influenza and parainfluenza viruses	Encephalitis	A	Diagnosis suggested by CSF molecular testing
JC virus	Progressive multifocal leukoencephalopathy	A	Molecular method is the test of choice. False positive result may happen due to high-level of BKV
LCMV	Meningoencephalitis	B ^b	Serology is the test of choice. Molecular method may enhance sensitivity
Measles virus	Sub-acute sclerosing panencephalitis	C	CSF antibodies, CSF index, brain tissue PCR
Mumps virus	Encephalitis, meningitis	B	Serology, throat swab PCR, CSF culture, or PCR
Nipah and Hendra viruses	Meningitis, encephalitis	C	Serology (special pathogen branch, CDC)
Parvovirus	Encephalitis	B	Serum and CSF IgM/IgG in combination with serum and CSF PCR is the test of choice
Rabies virus	Encephalitis	B ^b	Antibodies (serum, CSF), PCR of saliva, skin, or CSF, IFA of nuchal biopsy, or CNS tissue. Coordinate testing with local health department
VZV	Meningitis, myelitis	B	CSF PCR and CSF serology are indicated
West Nile virus	Encephalitis, myelitis	B ^b	CSF IgM, paired serology (cross-reactivity with other flaviviruses). CSF PCR limited to diagnosis in immunocompromised patients who have impaired humeral response
Zika virus	Microcephaly	A	PCR has been used for plasma, urine, and CSF. Serology is useful
Bacteria			
Gram-negative rods, mainly <i>E. coli</i>	Meningitis	A-B	Bacterial culture is the test of choice. Commercial molecular device is available
<i>Listeria monocytogenes</i>	Meningitis, encephalitis	A-B	Bacterial culture is the test of choice. Commercial molecular device is available
<i>Neisseria meningitidis</i>	Meningitis	A-B	Bacterial culture is the test of choice. Commercial molecular device is available
Group B <i>Streptococcus</i>	Meningitis	A-B	Bacterial culture is the test of choice. Commercial molecular device is available

Table 3 (continued)

Organisms	Clinical diseases/disorders	Molecular test applicability ^a	Comments
<i>Haemophilus influenzae</i>	Meningitis	A-B	Bacterial culture is the test of choice. Commercial molecular device is available
<i>Mycobacterium tuberculosis</i>	Meningitis, myelitis	A-B	CSF mycobacterial culture remains the gold standard, but commercial molecular device is available
<i>Nocardia</i> species	Encephalitis	C	Bacterial culture is the test of choice. Usefulness of molecular methods needs to be determined
<i>Ehrlichia</i> species	Encephalitis	A-B	Morulae in white blood cells, PCR of whole blood, paired serology. CSF PCR may be positive but less sensitive than testing of whole blood
<i>Streptococcus pneumoniae</i>	Meningitis	A-B	Bacterial culture is the test of choice. Commercial molecular device is available
<i>Treponema pallidum</i>	Neurosyphilis, myelitis	C	CSF VDRL, serum RPR with confirmatory FTA-ABS
<i>Borrelia burgdorferi</i>	Lyme disease	B	Serology is the test of choice. Molecular method available
<i>Bartonella</i> species	Encephalitis	B	Serology (acute usually diagnostic), PCR of lymph node, CSF PCR not useful
<i>Tropheryma whippelii</i>	Whipple disease, lymphadenopathy	A	CSF PCR, PAS-positive cells in CSF, small bowel biopsy
Fungi			
<i>Cryptococcus neoformans</i>	Encephalitis, meningitis	C	Antigen detection is the test of choice. Culture is useful. Molecular method has potential
<i>Histoplasma capsulatum</i> , <i>B. homini</i> , and <i>C. immitis</i>	Encephalitis, meningitis	C	Culture is the test of choice. Values of molecular method are to be determined
<i>Aspergillus</i> species	Encephalitis	C	Culture is the test of choice. Histopathology and serum/CSF galactomannan assay are helpful. Molecular method has potential
<i>Zygomycetes</i> species	Encephalitis	C	Culture is the test of choice. Histopathology is helpful. Molecular method has potentials
Parasites			
<i>Toxoplasma gondii</i>	Encephalitis	B	Serology is the most useful diagnostic test. False negative serology results may happen in immunocompromised hosts. Values of molecular tests on CSF need to be determined
Free-living amoeba, e.g., <i>Acanthamoeba</i> and <i>Balamuthia</i>	Encephalitis	C	Molecular method may provide specific confirmed diagnosis

HSV herpes simplex virus, CMV cytomegalovirus, HHV human herpesvirus, CSF cerebrospinal fluid, HTLV human T cell lymphotropic virus, LCMV lymphocytic choriomeningitis virus, VZV varicella zoster virus

^a A, test is generally useful for the indicated diagnosis; B, test is useful under certain circumstances or for the diagnosis of specific forms of infection, as delineated in the right-hand column; and C, test is seldom useful for general diagnostic purposes but may be available in reference laboratories for epidemiological studies or for the diagnosis of unusual conditions

^b Molecular methods were used in these cases associated with transplant of solid organ from infected donor

for JEV infection as well as therapeutic targets for anti-JEV therapy [89, 90]. Host neural epidermal growth factor like 2 and apolipoprotein B in CSF were able to diagnose tuberculous meningitis with 83.3–89.3 % sensitivity and 75–92 % specificity [91, 92]. CSF metabolite profiling has been reported useful in classification, diagnosis, epidemiology, and treatment assessment of CNS infections in HIV patients [93–95]. CSF metabolic profile analysis implicated bioenergetic adaptation as a neural mechanism regulating shifts in cognitive states of HIV-infected patients [96].

Selective Testing Results to Interpret Correlation with Clinical Diseases

Identification of an etiologic agent in patients with CNS infections requires consideration of the most likely causative organisms, the available diagnostic tests for these agents, and the highest-yield clinical specimens for testing. Knowledge of the epidemiology and clinical presentation of specific agents is critical in selecting which diagnostic methods are appropriate for a given patient. In particular, animal or vector exposures,

geographic location, recent travel history, season of the year, exposure of ill contacts, and occupational exposures should be considered.

When selecting appropriate pathogen-specific molecular diagnostic methods, the following factors should be considered. CSF is the optimal specimen for PCR testing for patients with suspected meningitis or meningoencephalitis. While indirect evidence can be gained by testing of other specimen types, attempts should be made to obtain CSF samples early before commencement of treatment that can compromise yield. Time of testing from symptom onset is essential to understand and rule out false negative results and recommend retesting within a certain time frame. For example, HSV PCR can commonly render false negative results if CSF sample is obtained very early or late in the process of HSE infection. Host health condition is known to influence the test performance characteristics. Immunocompromised patients are at risk for infection by a much wider array of opportunistic pathogens, for example, HHV-6, JC virus, *Toxoplasma* encephalitis in bone marrow transplant recipients, and patients with HIV. Often, infection can be more severe (e.g., WNV) and difficult to diagnose in this population. Table 3 provides the practical recommendations on application and pitfalls of molecular test for diagnosis of CNS infections.

Finally, a positive nucleic acid amplification testing result is complicated by the fact that some viruses survive latently in macrophages or neurologic tissues and are incidentally detected by sensitive molecular techniques without an actual pathogenic role and can potentially lead to overtreatment. Uses of adjunctive biomarkers that depict active replication are being explored to overcome this drawback.

Conclusion

Historically, identification of microbiologic agents in patients with CNS infections has been hindered by the low yield of CSF culture for viral and fastidious bacterial organisms, delays in CNS production of organism-specific antibodies, and difficulties in obtaining optimum samples for testing. Nucleic acid in vitro amplification-based molecular diagnosis methods have a wider and better application in clinical microbiology practice. The monoplex assay will likely be the main platform for urgent, random-access, low-throughput assays. Multiplex assays have the additional advantage of detecting multiple targets and mixed infections. As volume of CSF sample retrieved is often small, multiplex assays enable comprehensive diagnostic analysis with low amount of sample, obviating need for repeated lumbar punctures. The clinical relevance and cost-effectiveness of simultaneous multipathogen detection and identification strategies merit further investigation. Application of pan-omic techniques in difficult-to-diagnose CNS infections is the new exciting frontier; the technology

is promising, but routine implementation is expected to be slow due to various challenges such as lack of applicable regulatory guidelines and adaptation in the clinical setting.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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