


Diagnostic Testing in Central Nervous System Infection

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Abstract

Patients with central nervous system (CNS) infection experience very high levels of morbidity and mortality, in part because of the many challenges inherent to the diagnosis of CNS infection and identification of a causative pathogen. The clinical presentation of CNS infection is nonspecific, so clinicians must often order and interpret many diagnostic tests in parallel. This can be a daunting task given the large number of potential pathogens and the availability of different testing modalities. Here, we review traditional diagnostic techniques including Gram stain and culture, serology, and polymerase chain reaction (PCR). We highlight which of these are recommended for the pathogens most commonly tested among U.S. patients with suspected CNS infection. Finally, we describe the newer broad-range diagnostic approaches, multiplex PCR and metagenomic sequencing, which are increasingly used in clinical practice.

Keywords

- ▶ meningitis
- ▶ encephalitis
- ▶ diagnostic
- ▶ metagenomic sequencing

Inflammatory syndromes of the central nervous system (CNS) such as meningitis, encephalitis, and myelitis confer very high morbidity and mortality. CNS inflammatory syndromes can have both infectious and noninfectious causes. Distinguishing between these is critical to guide treatment, but the presence or absence of infection cannot be clearly determined based on symptoms, physical examination, or routine laboratory and imaging studies. Clinicians must therefore rely on microbiological tests to identify a potential causative pathogen, and this is often a challenging endeavor. Patients can be highly symptomatic but have an extremely low organism burden. Optimal diagnostic testing strategies differ between pathogens, reflecting diverse mechanisms of infection and variability in immune responses. Multiple factors affect test performance, including disease prevalence and nuances of the specific platform.


These complexities often lead clinicians to utilize a patchwork of tests, each with their own strengths and limitations. Traditional microbiologic methods such as culture have low sensitivity but can be useful for the diagnosis of bacterial and

fungal infections. Molecular assays such as the polymerase chain reaction (PCR) are specific and have improved sensitivity relative to culture. Serologic tests are useful for detecting the immune response against a pathogen, but distinguishing past from current infection can be a challenge. Both PCR and serology require clinicians to choose targeted pathogens. Newer diagnostic modalities such as the Biofire FilmArray meningitis/encephalitis syndromic panel (FA-MEP, bioMerieux, Marcy-l'Etoile, France) and metagenomic sequencing allow testing for multiple pathogens simultaneously, but are expensive and relatively low-throughput in their current form. Here, we review these techniques and their application to commonly tested pathogens among U.S. patients with suspected CNS infection.

General Approach to Diagnosing CNS Infection

Developing a Diagnostic Strategy

Several key points should guide providers managing a patient with suspected CNS infection. The first is the importance of developing a plan for the diagnostic workup. Clinicians are recommended to first test for the most likely and

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“can’t miss” pathogens for a given patient, knowing the optimal test to use for each pathogen. If these first-line tests are unrevealing, saved cerebrospinal fluid (CSF) can be used for follow-up testing. The diagnostic plan should be in place prior to obtaining a lumbar puncture (LP), so that all desired first-line tests are sent, and no extraneous tests use precious CSF. Specific recommendations from the International Encephalitis Consortium include: record the LP opening pressure; collect at least 20 cc of CSF; save 5 to 10 cc for future testing; and test all CSF samples for glucose (along with paired peripheral glucose), protein, white blood cell (WBC) count with differential, and red blood cell count.¹ The importance of collecting a large volume of CSF and asking the clinical laboratory to save a portion of it cannot be underestimated. This facilitates additional workup after the most common causes of infection are ruled out, and prevents the need for a repeat LP and interpretation of results confounded by anti-infective and anti-inflammatory treatments.

In addition to CSF studies, it is important to gather corroborating evidence for CNS infection from peripheral sites, both broadly (e.g., with blood cultures) and in a directed fashion (e.g., with serologies).² Serum human immunodeficiency virus (HIV) testing is particularly important to consider, since the differential diagnosis for CNS infection is broader in immunocompromised patients. Baseline serum should also be stored for future testing, since some infections are diagnosed by testing acute and convalescent (4–6 weeks) sera. Above all, close bidirectional communication with the clinical pathologists in the microbiology and chemistry laboratories is paramount to ensure that the optimal diagnostic assays are chosen and that tests are followed up in a timely fashion.

Nonspecific CSF Studies

Chemistry and Cell Counts

The value of CSF chemistry and cell counts lies primarily in their ability to rapidly establish the presence or absence of CNS inflammation, though caution should be taken in interpreting these tests in immunocompromised patients who may not have markers of neuroinflammation. The classic teaching that viral pathogens lead to a predominantly lymphocytic pleocytosis while bacterial etiologies result in a neutrophilic predominance and low glucose is an oversimplification of a dynamic process with significant overlap. For example, in a pediatric series of 158 patients, neutrophilic predominant CSF had a positive predictive value of 81% and a negative predictive value of just 3% for distinguishing bacterial from viral meningitis.³ Conversely, 8 out of 82 patients with meningococcal meningitis had a normal CSF WBC count.⁴ Despite this, certain patterns can be useful to guide further testing, particularly when first-line testing is uninformative. Examples include the moderately low CSF glucose levels typical of mumps and lymphocytic choriomeningitis virus (LCMV), and the persistent neutrophilic pleocytosis found with West Nile virus (WNV) and cytomegalovirus (CMV).⁵ An eosinophilic pleocytosis should raise concern for helminth infections. The thermally dimorphic fungus

Coccidioides has also been described to cause mild elevation in CSF eosinophils.⁶

Gram Stain and Culture

Whether pleocytosis is present or not, any patient undergoing an LP due to concern for infection should have CSF Gram stain and culture performed. The sensitivity of Gram staining depends on the organism burden, ranging from 25% for *Listeria monocytogenes* to upwards of 90% for *Streptococcus pneumoniae*. Sensitivity drops by 50% if patients have already received antibiotics.⁷ A positive Gram stain is highly specific for bacterial meningitis.

The specificity of bacterial culture is also high, nearly 100%; however, the sensitivity varies by pathogen and decreases with increasing interval between antibiotic exposure and LP. *Neisseria meningitidis* is sterilized within 2 hours after treatment, while *S. pneumoniae* can be detected up to 8 hours after initiation of treatment, albeit with low sensitivity.^{8,9} The sensitivity of culture for tuberculous meningitis is estimated to be 60%, but can increase to >85% by performing up to four large-volume (10–15 mL) LPs.¹⁰ The primary drawback is that results are not provided in a clinically actionable timeframe due to the slow growth rate of mycobacteria.^{11,12}

Pathogen-Specific Testing by Modality

Because only a small number of pathogens can be diagnosed using Gram stain and culture, clinicians must rely heavily on pathogen-directed testing. This is challenging because there are several different types of testing modalities that can be used (→Fig. 1), combined with a multitude of potential pathogens. →Table 1 summarizes the preferred and alternative diagnostic tests for common pathogens, discussed in greater detail below. Unfortunately, there are no streamlined rules-of-thumb to guide the selection of particular tests for particular pathogens. It can help to understand the pathophysiology of different organisms causing CNS disease. In general, when choosing a pathogen-specific test, clinicians must take into account:

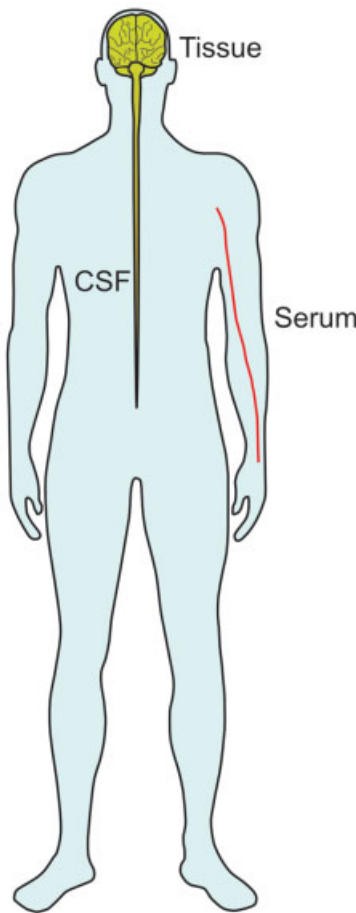
- What pathogen is being interrogated;
- How to test for it (e.g., serology vs. PCR);
- Where to look (e.g., CSF vs. blood);
- When to test (e.g., need for paired acute/convalescent sera).

Another important consideration is the turnaround time between ordering a test and receiving its results, since clinical decisions often need to be made empirically while awaiting test results. In general, PCR tests are rapid and often available in-house, while specialized serology tests may only be available at reference laboratories. However, there is substantial variation between clinical microbiology laboratories, so close communication with laboratory personnel is essential.

Detection of Immune Response by Serology

Serological tests are widely used in the diagnosis of CNS infections, particularly for pathogens present at low levels or for brief periods of time, and for diseases whose manifestations are primarily mediated by the host antibody response.

A. SPECIMEN TYPES



B. DIAGNOSTIC MODALITIES

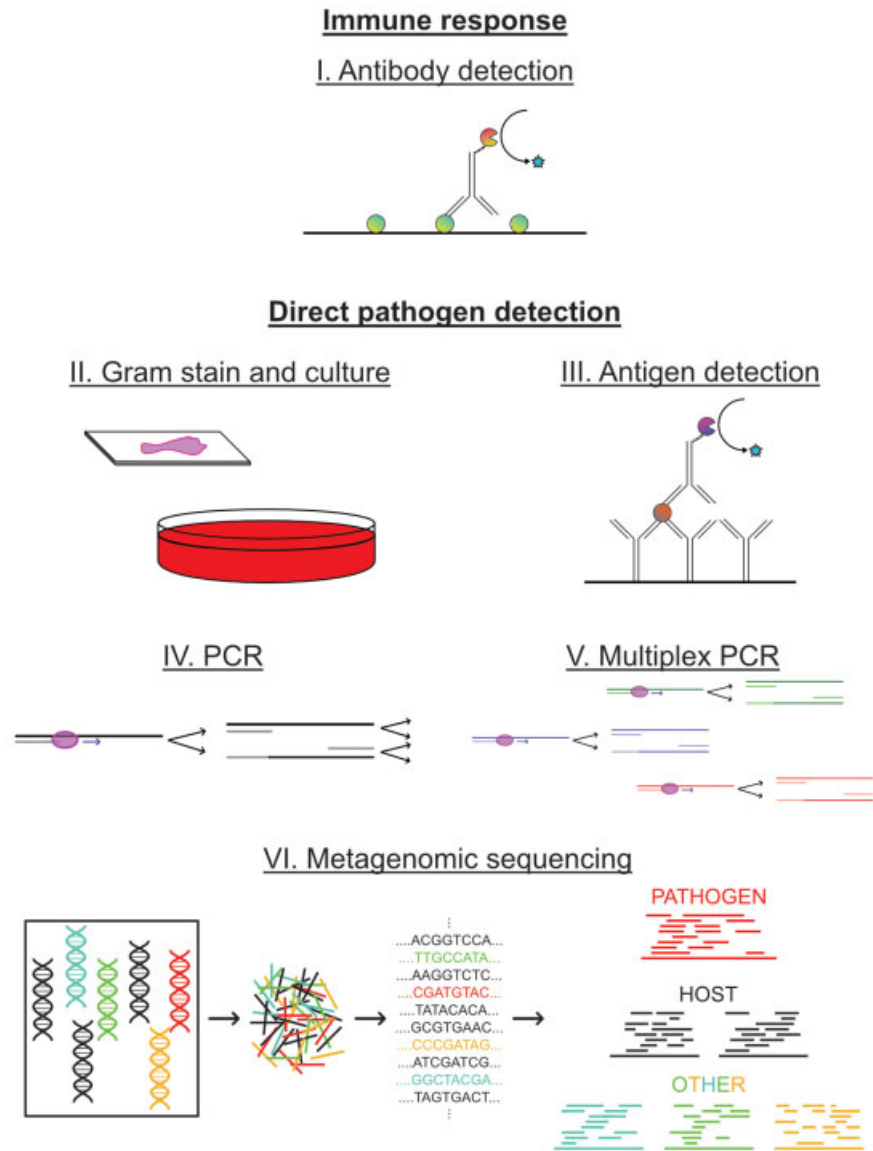


Fig. 1 Overview of specimen types and modalities for diagnosing CNS infection. Panel (A) represents key anatomic compartments for sampling. Panel (B) provides an overview of the major diagnostic modalities. (I) Indirect evidence of infection can be provided by pathogen-specific antibody responses. Illustrated is a generic ELISA. (II) Gram stain and culture are performed for bacterial and fungal pathogens. (III) Pathogen antigen detection is most often performed by a modification of the ELISA assay. (IV) PCR detects nucleic acid from a prespecified target. (V) Multiplex PCR expands (IV) to examine a set of predetermined targets. (VI) Metagenomic sequencing amplifies all nucleic acid directly from a biological sample, including bacteria, fungi, and viruses, as well as human nucleic acid and microbes present in reagent or laboratory environment. Bioinformatic analyses are used to identify potential pathogens. CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

There are several considerations in interpreting the presence or absence of antibodies in CSF. For acute infection, CSF immunoglobulin M (IgM) is generally diagnostic of intrathecal antibody production, since IgM does not cross the blood–brain barrier well, although blood introduced into the CSF sample during LP can lead to false-positive results. Because IgM antibodies can be cross-reactive for viruses within the same family (e.g., flavivirus), serology is generally conducted in two steps. The first is often screening with an enzyme immunoassay (EIA), in which a patient’s sample is allowed to bind to an immobilized antigen, then a secondary antibody

targets the immunoglobulin. IgM immunoglobulins are often captured first to reduce background from potentially cross-reactive immunoglobulin G (IgG). Samples positive by screening EIA undergo confirmatory testing, e.g., with a plaque reduction neutralization test (PRNT), in which serial dilutions of patient sample are mixed with virus then incubated with cell culture to look for plaque formation.¹³ In contrast to IgM, IgG does cross the blood–brain barrier, so diagnosis of chronic or reactivated infections requires demonstration of an elevated CSF:serum IgG ratio, normalized to the CSF:serum ratio of albumin. Testing antibody

Table 1 Summary of common CNS pathogens and the diagnostic tests available

Pathogen	Common CNS syndromes	Risk factors	Preferred diagnostic test	Alternative and supportive diagnostic test(s)
Adenovirus	Encephalitis	Immunocompromise	CSF PCR	PCR from respiratory tract
CMV	Encephalitis	Immunocompromise	CSF PCR	
EBV	Encephalitis	Immunocompromise	CSF PCR	Serology
HSV-1	Encephalitis		CSF PCR	
HHV-6	Encephalitis	Immunocompromise	CSF PCR	Serum PCR (rule out chromosomal integration)
Rabies virus	Encephalitis	Exposure to infected mammal	PCR from skin or brain biopsy	Acute and convalescent serology
<i>Bartonella</i>	Encephalitis		Serology	PCR
<i>Toxoplasma</i>	Encephalitis	Immunocompromise	Serology and CSF PCR	
VZV	Encephalitis and meningitis	Immunocompromise	CSF PCR and serology	
West Nile virus (and other arboviruses)	Encephalitis and meningitis	Exposure to mosquito	CSF IgM	Serum IgM, acute and convalescent serology, CSF PCR
Mycoplasma	Encephalitis and meningitis		PCR from CSF and respiratory tract	Acute and convalescent serology
<i>Brucella</i>	Meningitis and encephalitis	Exposure to animals or unpasteurized dairy	CSF serology	
<i>Listeria</i>	Meningitis and encephalitis	Immunocompromise, including mild exposure to unpasteurized dairy	CSF gram stain and culture	CSF PCR Blood culture
Enterovirus	Meningitis		CSF PCR	PCR from respiratory tract or stool
HIV	Meningitis		CSF PCR	
HSV-2	Meningitis		CSF PCR	
LCMV	Meningitis	Exposure to house mice excreta	CSF PCR	Acute and convalescent serology
<i>Parechovirus</i>	Meningitis	Children	CSF PCR	
<i>Borrelia</i>	Meningitis	Exposure to tick	Serology: serum and CSF	
<i>Cryptococcus</i>	Meningitis	Immunocompromise	CSF antigen	
<i>Leptospira</i>	Meningitis	Water and soil exposure	Acute and convalescent serology	PCR
JC virus	Other	Immunocompromise	CSF PCR	
<i>Treponema</i>	Other		Serology: serum and CSF	

Abbreviations: CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IgM, Immunoglobulin M; JC, John Cunningham; LCMV, lymphocytic choriomeningitis virus; PCR, polymerase chain reaction; VZV, varicella zoster virus.

responses from the serum can also aid in diagnosis, but must be interpreted carefully, since prior vaccination or treatment with blood products can lead to false-positive results. In some cases, IgG avidity can be used to distinguish recent infection (low avidity) from prior infection or vaccination (high avidity). Finally, in interpreting results from serological tests, it is critical to consider factors that can lead to false-negative results such as treatment with B cell depleting immunotherapy.

Viruses Generally Diagnosed by Serology

The serological techniques described above are essential to establishing the diagnosis of RNA arthropod-borne viruses (arboviruses), the most common of which in the United States is WNV. CNS manifestations of WNV include encephalitis, meningitis, and acute flaccid myelitis (AFM). WNV replication in blood and CSF often precedes the onset and evaluation of neurological symptoms.¹⁴ Therefore, WNV PCR from CSF is generally unreliable for establishing the

diagnosis; it has a reported sensitivity of 57%,¹⁵ though may be higher in immunocompromised patients.¹⁶ Instead, WNV is usually diagnosed by serology, with a testing algorithm comprised of screening EIA followed by confirmatory PRNT. Testing can be performed from CSF or serum, and per Centers for Disease Control and Prevention (CDC) guidelines,¹⁷ establishing that a diagnosis of neuroinvasive WNV requires one of the following:

- Virus-specific IgM antibodies in CSF, and negative CSF IgM for other endemic arboviruses;
- Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies;
- Paired acute and convalescent serum demonstrating a fourfold or greater change in virus-specific antibody titers;
- Isolation of virus or demonstration of specific viral antigen or nucleic acid.

An important consideration in diagnostic testing for WNV is that IgM, which arises 3 to 9 days after the onset of illness, can persist for months to years.¹⁸ Therefore, finding a positive IgM is not as indicative of recent infection for WNV as it is for other pathogens.

There are a variety of other domestic arboviruses. Those transmitted by mosquitoes include the California serogroup (California encephalitis virus, Jamestown Canyon virus, Snowshoe Hare virus, and La Crosse virus, which is most common in children); Eastern equine encephalitis virus; and St. Louis encephalitis virus. Tick-borne encephalitis viruses in the United States include Powassan virus and Colorado tick fever virus, the latter of which is more common in children. For details regarding other neurotropic arboviruses worldwide, readers are referred to Venkatesan et al.¹ The most common include Japanese encephalitis virus (JEV) and tick-borne encephalitis virus. All neurotropic arboviruses are generally diagnosed by serology using the same criteria described for WNV, though PCR may be more useful for some, e.g., JEV.¹⁶

Bacteria Generally Diagnosed by Serology

Brucellosis is a zoonotic infection caused by Gram negative bacteria from the genus *Brucella*. Up to 5% of patients with brucellosis have CNS symptoms including meningitis, encephalitis, psychiatric disorders, sensorineural hearing loss, neuropathies, radiculitis, and myelitis.^{16,19} The gold standard for diagnosis is serology using the serum agglutination test (SAT). Originally developed in 1897,²⁰ the SAT has a sensitivity of 96% and specificity of 100% in nonneurologic *Brucella* infection.²¹ In patients with neurobrucellosis, the SAT from CSF has a sensitivity of 79 to 94%,^{19,22} and a specificity of 96%.¹⁹ Although the SAT is the gold standard for diagnosis of neurobrucellosis, it is limited by its complexity, decreased sensitivity early in infection, cross-reactivity with other gram-negative pathogens, difficulty in interpretation in areas of high prevalence, and distinguishing prior versus chronic infection.²³ CSF enzyme-linked immunosorbent assay (ELISA) is often used as an alternative, and has a sensitivity ranging between 80 and 100% and a specificity of 98%.^{22,24} The sensitivity of CSF culture is low, ranging from 15 to 25%.^{19,22} Diagnosis can also be made by blood or bone

marrow culture, but sensitivities range from 50 to 90% in acute infection and decrease over time.²⁵ PCR assays have also shown promising results^{23,26} but have not yet been validated against serologic tests. Given that *Brucella* is highly contagious, clinicians should notify the microbiology laboratory to ensure that proper biosafety measures are taken.

Bartonella henselae is a gram-negative bacillus that causes cat-scratch disease after transmission from asymptomatic bacteremic cats. Approximately 1 to 10% of patients with cat-scratch disease develop neurologic manifestations, primarily lymphocytic meningoencephalitis²⁷ and neuroretinitis characterized by the sudden onset of unilateral loss of vision and stellate macular exudates on funduscopy.¹⁶ Infection in HIV patients can on occasion lead to neuropsychiatric disorders.²⁸ Serologies are the mainstay of diagnosis for *B. henselae* infection, but different methods have widely varying performance. ELISA has a sensitivity between 10 and 71%, depending on the preparation technique and whether IgG or IgM is tested.²⁹ PCR assays^{30,31} are available at reference laboratory centers. The performance of serologies and PCR in patients with CNS infection is under-explored but appears equivalent to those with more common manifestations.^{28,32}

Spirochetal Bacteria Generally Diagnosed by Serology

Treponema pallidum, which causes syphilis, can involve the CNS during both secondary and tertiary (late) stages.¹⁶ CNS manifestations of secondary syphilis include headache, meningismus, asymmetric paraparesis, hyperreflexia, neurogenic bladder, visual and hearing loss, and facial weakness. Late neurosyphilis can present as meningovascular involvement with seizures and a stroke-like syndrome, or parenchymal involvement with tabes dorsalis and general paresis.³³ Atypical presentations include a temporal lobe encephalitis that can mimic herpes simplex virus (HSV) encephalitis.³⁴ Syphilis is diagnosed by serology. First, screening is performed using a serum treponemal test: fluorescent treponemal antibody absorption (FTA-ABS), *T. pallidum* particle agglutination (TP-PA), or an antitreponemal ELISA. All of these are useful for diagnosing syphilis at any stage but remain positive for life, regardless of treatment. If negative, the diagnosis of neurosyphilis can be ruled out. If positive, nontreponemal tests are used to confirm active infection and monitor treatment response. The Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin tests assess for antibodies that react with endogenous antigens (lecithin, cholesterol, and cardiolipin). Neurosyphilis can be diagnosed by CSF VDRL, which has a specificity of 99.8% but a sensitivity of only 30 to 68%.^{35,36} Other tests performed from CSF have low sensitivity, including PCR³⁷ and FTA-ABS and ELISA,³⁸ though TP-PA may be more promising.³⁹ Therefore, even if CSF VDRL is negative, patients with positive syphilis serology, CSF pleocytosis, and compatible symptoms can be treated presumptively for neurosyphilis.

Borrelia burgdorferi causes Lyme disease, including neuroborreliosis. Early neuroborreliosis symptoms in the United States include lymphocytic meningitis, seventh nerve cranial neuritis, and painful radiculoneuropathy. Most patients clear CNS infection without treatment⁴⁰ but a small percentage

develop late Lyme neuroborreliosis months to years later. This syndrome is defined as continuous symptoms occurring for >6 months and is characterized by chronic meningitis, encephalitis, myelitis, and cerebral vasculitis.⁴¹ Both early and late neuroborreliosis are diagnosed by demonstrating intrathecal antibody production using CSF to serum antibody index⁴² in patients with CSF pleocytosis. The antibody index has a sensitivity of >95% in patients with at least 6 weeks of symptoms, but only 74% in patients with less than 6 weeks of symptoms.⁴³ Therefore, if initial testing is negative and there is high clinical suspicion, the antibody index should be repeated. Nearly all patients with neuroborreliosis have positive serum Lyme testing, so if this is negative, alternative diagnoses should be considered or Lyme serology should be repeated. CSF PCR has low sensitivity and is not routinely used. Results of CSF antibody testing should be interpreted with caution⁴⁴: testing should not be performed in patients without compatible symptoms given the potential for false positives in areas of endemicity,⁴⁵ and the antibody index can remain elevated for years after successful treatment.⁴⁶

Leptospirosis is a zoonotic infection caused by *Leptospira* species in people who come into contact with urine or tissue from infected animals or contaminated environmental material, such as soil or water. The first phase of infection, attributed to hematogenous dissemination, is characterized by high fever, myalgias, and conjunctival suffusion. The second phase occurs up to 30 days later and can include aseptic meningitis, which is attributed to the immune response.¹⁶ Direct detection of the pathogen from blood and CSF is possible during the acute phase of illness by dark field microscopy, specialized culture, and PCR. However, in practice, diagnosis is made by serology, as the acute phase is usually not recognized. The gold standard for diagnosis is the microagglutination test (MAT). A fourfold increase in titers between acute and convalescent sera or a single titer of at least 1:800 in the setting of compatible symptoms is highly suggestive of infection. The sensitivity of MAT increases over time: 49% in the acute phase and 93% after day 30.^{47,48} The specificity is reported to be 97%, but is affected by cross-reactive antibodies to other spirochetes and viruses. An ELISA IgM is reported to have high sensitivity and specificity in low endemic areas,⁴⁷ but performs poorly in patients in the acute phase and those coming from endemic areas.⁴⁹ Early results for a modified lateral flow assay have shown promise for rapid diagnosis including possibly early meningitis.⁵⁰

Fungi Generally Diagnosed by Serology

Meningitis is a serious complication of disseminated infection by the endemic fungi *Coccidioides immitis*, *Coccidioides posadasii*, and *Histoplasma capsulatum*. CSF culture is positive in only 20 to 65% of cases, making serologies the mainstay of diagnosis.^{51–53} IgM and IgG can be detected in CSF using a two-tiered test: first with EIA, which is sensitive but relatively nonspecific, followed by confirmatory complement fixation or immunodiffusion, which are specific but insensitive.^{54,55} Several recent studies highlight the potential use of antigen for direct detection of pathogen,^{54,56} but these assays are currently not U.S. Food and Drug Administration (FDA)-approved.

Protozoa Generally Diagnosed by Serology

Toxoplasma gondii is a protozoan that humans can acquire through ingestion of undercooked meats, through handling contaminated cat litter, or transplacentally. With the exception of congenital toxoplasmosis, manifestations of CNS illness primarily occur due to reactivation of latent infection in patients with defects in T-cell-mediated immunity such as acquired immunodeficiency syndrome (AIDS), hematologic malignancy, exposure to antitumor necrosis factor therapies, and solid organ transplants. The most common CNS presentations are encephalitis and chorioretinitis. The first step in establishing *T. gondii* infection is to determine the patient's serostatus. Negative IgG makes toxoplasmic encephalitis extremely unlikely.⁵⁷ If IgG is positive and imaging studies are consistent with toxoplasmosis (i.e., multiple ring-enhancing lesions), the patient can be treated empirically.¹⁶ If imaging shows a single lesion or the patient clinically worsens despite treatment, CSF PCR is recommended. The sensitivity of CSF PCR ranges between 33 and 75%,^{58,59} so if negative and clinical suspicion remains high, it may be necessary to pursue biopsy.

Direct Detection of Pathogen or Antigen

Microscopic examination (CSF wet mount) is useful for protozoal infections including *amebic meningoencephalitis*.⁶⁰ *Naegleria fowleri* causes primary amebic meningoencephalitis in individuals with warm freshwater exposure. *Balamuthia*, and less commonly, *Acanthamoeba*, causes the more indolent granulomatous amebic meningoencephalitis. In addition to direct visualization by microscopy, specialized testing such as tissue PCR and immunohistochemistry are also available through the CDC. East African *trypanosomiasis*, which causes sleeping sickness, is diagnosed by direct visualization of trypanosomes in CSF, brain tissue, or blood. By contrast, serology is used to diagnose American trypanosomiasis, which causes Chagas disease, including rarely meningoencephalitis.

Helminth infections can also occasionally be detected by direct visualization. *Angiostrongylus cantonensis* (the rat lungworm) causes acute eosinophilic meningitis after ingestion of snails or other contaminated food; though historically most common in the tropics, this infection is increasingly detected in the United States. Other helminth infections associated with eosinophilic meningitis include *Baylisascaris*, *Gnathostoma*, *Paragonimus*, *Toxocara*, and *Schistosoma*. All of these are rarely identified by direct visualization in CSF, and diagnosis often involves a combination of presumptive clinical diagnosis and serology (reviewed in Walker and Zunt⁶⁰ and Graeff-Teixeira et al⁶).

Direct detection of microbial antigens, comprised of proteins and glycoproteins, can occasionally be useful. A good example is the cryptococcal antigen test (CrAg). *Cryptococcus neoformans* causes meningitis in patients with defects in cell-mediated immunity, including AIDS, solid organ transplant, advanced malignancy, prolonged exposure to high-dose steroids, and sarcoidosis.¹⁶ The related *Cryptococcus gattii* causes meningitis in immunocompetent patients. Both are detected by CrAg, which is directed at

the polysaccharide capsule. This test is highly sensitive and specific; commercially available assays have positive and negative predictive values between 92 and 100%.⁶¹ While the initial titer has prognostic value, it cannot be followed serially to monitor treatment response.^{62,63} For patients in whom LP is not accessible or contraindicated, the World Health Organization (WHO) recommends substituting with a serum CrAg assessed on a lateral flow assay or a latex agglutination platform. Serum CrAg has a sensitivity of 96 to 100% and a specificity of 98 to 100% relative to CSF CrAg and culture.⁶¹

Several rapid antigen-based assays for bacterial meningitis were introduced at the turn of this century but have had disappointing performance.^{64,65} With the advent of molecular techniques, this approach has largely fallen out of favor.

Direct Detection of Pathogen Nucleic Acid by PCR

PCR detects pathogen nucleic acid by amplifying a short region of the genome using primers designed to match conserved nucleotide sequences. This method requires extraction of nucleic acid, which is often combined with amplification in a single assay. PCR can be performed directly from DNA, or can be used to detect RNA with the addition of a reverse transcription (RT) step (RT-PCR). This is distinct from real-time PCR, also known as quantitative PCR. While some clinical PCR tests are quantitative (e.g., HIV viral load), the PCR tests used to diagnose most CNS infections are qualitative, providing only a positive or negative result. PCR tests have been widely implemented in CNS diagnostics because they are highly specific, inexpensive, and much more rapid than culture (reviewed in Tunkel et al² and DeBiasi and Tyler⁶⁶). Many PCR tests have excellent sensitivity, but false-negative results can rarely occur due to inhibitors such as heme degradation products.⁶⁶ Individual PCR tests require only 30 to 200 μ L of sample⁶⁶; however, when multiple different tests are run, the total sample volume can be limiting.

DNA Viruses Generally Diagnosed by PCR

Many DNA viruses that cause CNS infection are human herpesviruses (HHVs), which are generally acquired early in life. CNS manifestations can occur with primary infection, but more frequently the virus becomes latent in neurons or other cells, and reactivation causes CNS disease. Diagnosis of CNS infection with herpesviruses therefore frequently depends on direct detection of viral DNA by PCR rather than serology, since many patients are seropositive at baseline.

HSVs are among the most common pathogens in CNS infection, and they affect immunocompetent patients. *HSV-1* (HHV-1) causes encephalitis, primarily involving the limbic region, and much less commonly causes meningitis. *HSV-2* (HHV-2) most commonly causes meningitis, which can be recurrent. Less commonly, HSV-2 can also cause encephalitis, primarily in neonates, as well as lumbosacral radiculitis and transverse myelitis.^{5,16} HSV-1 and HSV-2 are diagnosed by PCR, and can be tested separately, e.g., using type-specific primers, or together, e.g., using melting curve analysis.⁶⁶ The test characteristics of HSV-1 PCR have been extensively

studied, and overall show excellent sensitivity (96–98%) and specificity (94–99%),^{67,68} though sensitivity may be lower in neonates.⁶⁶ Sensitivity may also be low within the first 3 days of infection,⁶⁹ so Infectious Disease Society of America guidelines recommend repeating an LP with HSV PCR after 3 to 7 days if the first test is negative and there is high clinical suspicion.² Repeat testing is informative even after empiric treatment, since the sensitivity of HSV-1 remains high for the first 7 days after starting acyclovir.⁶⁷

Varicella zoster virus (VZV, HHV-3) causes chicken pox in primary infection and shingles in reactivation, and is associated with many CNS syndromes: postinfectious cerebellar ataxia in children with primary infection; myelitis associated with primary infection or reactivation; optic neuritis; vasculopathy; meningitis; and encephalitis.^{70,71} VZV encephalitis occurs most often in immunocompromised patients and can include necrotizing ventriculitis. In some cases, the pathophysiology of VZV encephalitis is believed to be due to small-vessel vasculopathy.^{66,71} VZV small-vessel vasculopathy can manifest as multifocal ischemic stroke, aneurysm, or hemorrhage. VZV can also cause large-vessel arteritis leading to stroke in immunocompetent patients, particularly after herpes zoster ophthalmicus.⁷² VZV vasculopathy can be difficult to diagnose because it can occur without typical dermatologic manifestations, or months after rash.⁷² Diagnosis of CNS VZV disease generally relies upon both PCR and serology.^{1,2} VZV PCR from CSF has variable sensitivity, ranging from 30% in patients with vasculopathy⁷² to 60% in patients with meningoencephalitis.⁶⁶ This may be because of delay between viral expression in CSF and the onset of neurological symptoms, and because some syndromes represent antibody-mediated disease.⁷² Therefore, VZV serology from CSF is an important additional test and can include CSF IgM for syndromes arising after primary VZV infection and CSF:serum IgG ratio for syndromes associated with VZV reactivation. Due to the complexity in testing for VZV, clinicians are recommended to discuss the optimal test (s), how to order them, and the expected turnaround time with the microbiology laboratory.

Epstein-Barr virus (EBV, HHV-4) causes encephalitis and meningitis in immunocompromised patients, and can also cause myelitis and cerebellitis as sequelae of primary infection in immunocompetent patients.^{5,66} EBV is diagnosed by CSF PCR, but finding EBV DNA in CSF is not conclusive evidence of disease, since EBV can also reactivate in the setting of another inflammatory process. Although this is overall rare,⁷³ it may occur more commonly in immunocompromised patients.⁷⁴ Therefore, for syndromes associated with primary EBV infection, it is recommended to also check peripheral serologies.¹ One compelling indication for PCR is distinguishing EBV-related CNS lymphoma from other mass lesions in immunocompromised patients; in this setting, EBV PCR from CSF is 100% sensitive and 98.5% specific.⁶⁶

CMV (HHV-5) causes encephalitis, which can be necrotizing and associated with hemorrhagic ventriculitis, as well as other CNS manifestations such as retinitis and lumbosacral radiculomyelitis.^{5,16} Diagnosis is made by PCR from CSF, which has a sensitivity of 82 to 100% and a specificity of

86 to 100% in immunocompromised patients.⁶⁶ Finding CMV in other tissues can support the diagnosis because CMV reactivation often involves multiple organs.

HHV-6 (most often HHV-6B) causes limbic encephalitis in immunocompromised patients, particularly those who have recently undergone stem cell transplantation. HHV-6 may also cause seizures in children during primary infection, though this is controversial.⁶⁶ Diagnosis of HHV-6 is made by PCR, but finding HHV-6 DNA in CSF is not conclusive evidence of disease. HHV-6 DNA has been found in 74% of brain tissue samples from asymptomatic individuals,^{72,75} and the positive predictive value of finding HHV-6 DNA in CSF is only 30%.² In part, this is because HHV-6, like other herpesviruses, is acquired early in life, establishes latent infection, and can reactivate, particularly in the setting of other infections. In addition, unlike other herpesviruses, HHV-6 can also integrate into the human genome.⁷⁶ This occurs within telomeres, and is called chromosomally integrated HHV-6 (ciHHV-6). Approximately 1% of the population have ciHHV-6 acquired at birth and expressed in every cell in their body, leading to asymptomatic high-grade HHV-6 expression. Therefore, if HHV-6 PCR from CSF is positive, it is also recommended to test whole blood for ciHHV-6, finding of which would argue against pathogenic HHV-6.^{5,77} Interpreting the results of a positive HHV-6 CSF PCR often requires judicious interpretation, taking into account the clinical and radiographic presentation, as well as the likelihood of alternative diagnoses.⁷⁸

A few nonherpes DNA viruses can also cause CNS infection. *Adenovirus* infects children and immunocompromised patients, particularly those who have undergone stem cell transplantation.⁵ Adenovirus causes encephalitis and less commonly meningitis, and is diagnosed by PCR from CSF. Positive PCR from a respiratory sample can also support the diagnosis.²

John Cunningham (JC) virus is a polyomavirus that is generally acquired early in life and remains latent in the kidneys. The virus can reactivate and invade the CNS in immunocompromised patients, in particular those with AIDS and those who receive natalizumab for multiple sclerosis. The classical CNS presentation of JC virus is progressive multifocal leukoencephalopathy, a syndrome of altered mental status and focal neurological deficits due to discrete brain lesions, which are most often bilateral and asymmetric, and develop in the periventricular and subcortical white matter. JC virus can also rarely cause meningitis, encephalopathy, and cerebellar granule cell neuronopathy.¹⁶ JC virus can be diagnosed by CSF PCR, which has a specificity of 100% but a sensitivity ranging between 58 and 92% depending on the clinical scenario; sensitivity is lower in HIV patients receiving antiretroviral therapy.¹⁶ Brain biopsy with immunohistochemistry and electron microscopy has higher yield, but is obtained less frequently. Other noninvasive tests are generally not useful; 55 to 85% of adults are seropositive for JC virus,¹⁶ and even PCR from serum or urine is positive in many immunosuppressed patients without CNS disease. Because of the risk of JC virus reactivation, risk stratification with JC virus serology and titer is recommended in patients receiving natalizumab.⁷⁹

RNA Viruses Generally Diagnosed by PCR

CNS infections caused by RNA viruses generally occur as a manifestation of primary infection, since RNA viruses do not become latent or reactivate. For some RNA viruses, PCR can be used to detect viral RNA in CSF, while others are best diagnosed using serology because of a short period of viral replication.

Enteroviruses are a very common cause of aseptic meningitis, and can also cause encephalitis, notably recurrent meningoencephalitis in children with hypogammaglobulinemia. This highly diverse group is comprised of multiple strains of enteroviruses (EVs), as well as echoviruses, coxsackieviruses A and B, and poliovirus. Nonpolio EVs are diagnosed using RT-PCR with an assay that targets a conserved region of the viral genome in the 5'-untranslated region.⁶⁶ Enterovirus PCR has excellent sensitivity and specificity, both of which are overall estimated to exceed 95%.⁶⁶ An exception to this is EV71, which is much less reliably detected by CSF PCR; in one case series, only 31% of CSF samples were positive.⁸⁰ In cases with high suspicion for enterovirus and negative CSF PCR, support for the diagnosis can also be achieved by enterovirus PCR testing from the respiratory tract and stool.²

Parechovirus, a related group in the picornavirus family, causes meningitis in young children, and is diagnosed by RT-PCR in a separate assay from enterovirus.

Other RNA viruses diagnosed by CSF PCR include *LCMV*, which causes meningitis and less commonly encephalitis in patients exposed to house mice.⁵ Acute and convalescent serology should be obtained if there is high suspicion for LCMV but a negative PCR. *HIV* has multiple CNS manifestations, including aseptic meningitis during acute infection, encephalitis due to the immune reconstitution inflammatory syndrome, and chronic dementia; these are diagnosed by CSF PCR,⁵ and quantitative viral load may be informative.⁶⁶ In unvaccinated individuals, *measles* can rarely cause encephalitis (1–6 months after infection) or subacute sclerosing panencephalitis, an insidious syndrome with an incubation period up to several years. Measles can be diagnosed by PCR from CSF or brain tissue, serology, or PCR or culture from the nasopharynx and urine.² *Mumps* can cause meningitis in unvaccinated individuals, and is diagnosed by CSF PCR, culture, or serology.²

Rabies virus causes an almost uniformly fatal encephalitis in patients who experience a bite or other exposure from a rabid mammal. The incubation period from exposure to onset of illness varies greatly due to the need for virus migration to the CNS from the peripheral nervous system at the site of exposure. The diagnosis of rabies is made by performing RT-PCR (or historically immunohistochemistry) from a biopsy, either from the brain itself or from the skin at the hairline at the nape of the neck.^{5,16} Additional diagnostic modalities include rabies RT-PCR from saliva, or rabies serology from CSF or serum; however, serology has low sensitivity and can be confounded if the patient received either vaccination or postexposure prophylaxis.

Bacteria for which PCR can Aid in Diagnosis

Although many bacteria are diagnosed by growth on CSF culture, PCR can be helpful for fastidious organisms or in cases where patients have received prior antibiotic treatment.

A good example is *Tropheryma whippelii*, an actinomycete bacteria that causes Whipple's disease and is difficult to culture. CNS manifestations of Whipple's disease include subacute altered mental status with memory impairment, ophthalmoplegia, and seizure, often in the setting of systemic gastrointestinal and joint symptoms. In general, Whipple's disease is detected by duodenal biopsy with periodic-acid Schiff stain, and it is recommended to obtain multiple biopsies to ensure that affected areas are not missed. CNS involvement is diagnosed by PCR from CSF or tissue, and it is recommended to use two assays targeting different genes to improve specificity.¹⁶

L. monocytogenes causes meningitis and meningoencephalitis in patients with even mild immune compromise, as well as rhombencephalitis with brainstem and cerebellar involvement in immunocompetent patients. *Listeria* is a gram-positive rod with a characteristic microscopic appearance that can be seen on Gram stain in approximately 30% of patients.^{81–84} CSF culture has a reported sensitivity of 80 to 90%,¹⁶ but in practice can be as low as 46%.⁸⁵ Blood culture can be informative if positive, but has variable sensitivity, ranging between 35 and 60%.^{81–83} CSF PCR is therefore an important additional test, especially for patients who have received prior antibiotics. In one study, listeria PCR was positive in nine out of nine CSF culture-positive cases, as well as five CSF culture-negative cases from patients who had received prior antibiotics.⁸⁶ In another study, CSF PCR was positive in 10 of 16 patients, including one patient with negative cultures from CSF and blood.⁸⁴

Mycoplasma pneumoniae can cause encephalitis, aseptic meningitis, transverse myelitis, acute disseminated encephalomyelitis and Guillain–Barre syndrome.¹⁶ Sensitivity of PCR from CSF has been reported to be 40 to 55%, and can be augmented by testing from respiratory tract specimens.^{87,88} However, the true performance of PCR is uncertain due to the lack of a standardized methodology and because many cases may reflect postinfectious immune-mediated disease. Therefore, serology can aid in the diagnosis, but requires both acute and convalescent samples since patients may have had prior infection.⁸⁹

In 2017, the WHO recommended the Xpert MTB/RIF Ultra assay (Cepheid, Sunnyvale, CA) as the test of choice for the diagnosis of meningitis due to *Mycobacterium tuberculosis*.⁹⁰ This test was shown in a large multinational prospective cohort study to have a sensitivity of 95% in definite TB meningitis, and a sensitivity of 70% for probable-to-definite disease.⁹¹

Broad-Range Diagnostic Testing Approaches

It can be quite challenging to identify the causative agent in a patient with suspected CNS infection using the standard approaches described above. A clinician must consider the likelihood of each potential pathogen and order the appropriate tests, while working with a limited volume of CSF. In addition to being time consuming, this approach is often ineffective; no etiology is identified in 60% of patients with encephalitis^{92–94} and meningitis.⁹⁵ Moreover, the cost of

such a workup has been estimated to be up to \$10,000 per patient.⁹⁶ Therefore, there is considerable interest in broad-range assays that offer the potential to identify multiple pathogens with a single test. Common examples of broad-range tests include amplification and sequencing of 16S for bacteria and internal transcribed spacer for fungi. While these have successfully been used in CNS infection, a significant limitation is that they do not detect viruses.

Multiplex PCR

Multiplex PCR offers an intermediate between targeted PCR tests and unbiased platforms by assessing for a predetermined set of pathogens highly associated with CNS infection. The BioFire FilmArray meningitis/encephalitis panel (FA-MEP) is a multiplex PCR assay that was FDA-approved in 2015 for the rapid diagnosis of community acquired CNS infection. The panel tests for six bacteria (*Escherichia coli* K1 capsular subtype, *Haemophilus influenzae*, *L. monocytogenes*, *N. meningitidis*, *Streptococcus agalactiae*, and *S. pneumoniae*), seven viruses (HSV-1, HSV-2, VZV, CMV, HHV-6, enterovirus, and parechovirus) and one fungus (*C. neoformans/gattii*). The assay requires 5 minutes of hands on time; it is a closed system that performs nucleic acid extraction and purification followed by two-stage amplification to increase specificity, and results are returned in 60 minutes. This platform has generated great interest due to its ease of use and rapid turnaround time, and a handful of studies have evaluated its performance.

Performance Compared with Traditional Tests

One large industry-funded multicenter study⁹⁷ and several small case series have investigated the performance of the FA-MEP relative to traditional single-plex PCR assays or culture. In a study by Leber et al, 1,560 prospective CSF samples were analyzed on the FA-MEP from pediatric (41%) and adult (59%) patients for whom LP and bacterial culture were clinically indicated.⁹⁷ Overall, 8.7% of samples were positive by FA-MEP, with more positive tests among pediatric patients (14%) than adults (5%). The FA-MEP results were concordant with traditional methods in 69.5% of positive samples, but had a false-positive rate of 68% for bacteria (12 of 15 were *S. pneumoniae*) and 22% for viruses. These values declined to 41 and 10% respectively on secondary adjudication. False negatives were rare (6/104) and occurred with samples containing enterovirus, HHV-6, and *S. agalactiae*. Negative predictive agreement, a measure of specificity used in the absence of a gold standard comparator, was high at >99.9%.

A recent retrospective study compared the performance of the FA-MEP to traditional tests for 291 positive CSF samples collected at the Mayo Clinic over 40 years.⁹⁸ Eighty (27.5%) of these samples contained bacteria. The overall positive predictive agreement (PPA), a measure of sensitivity used in the absence of a gold standard comparator, was 92.5% but varied by pathogen. PPA was >95% for enterovirus, HSV-2, CMV, VZV, HHV-6, *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*, but only 82.6% for HSV-1 and 54.6% for *Cryptococcus*. It is important to note that there were very few samples in either study with *L. monocytogenes*, *N. meningitidis*, *S. agalactiae*, *E. coli*, parechovirus, and CMV. Several other smaller retrospective case series

reinforce the value of FA-MEP in detecting viruses and highlight uncertainty for the identification of bacteria.^{99–102}

The use of the FA-MEP for suspected cryptococcal meningitis is not fully defined. The FA-MEP has high rates of false positivity for *Cryptococcus* in areas of low HIV prevalence^{97,98} but is reported to be as reliable as CSF cryptococcal antigen in areas of high HIV prevalence¹⁰³ and is highly concordant with quantitative culture. This latter characteristic offers a potential use case, where the FA-MEP could rapidly differentiate patients with recurrence of cryptococcal meningitis versus those with immune reconstitution in the setting of antiretroviral therapy.¹⁰³ It is important to note a case report of two nonimmunocompromised patients with false-negative results for *Cryptococcus* by FA-MEP.¹⁰⁴

Use in Clinical Practice

While the ease of use and rapid turnaround time are attractive features of the FA-MEP, its test characteristics indicate that it cannot yet replace traditional testing. The published literature suggests the primary role for this platform is to test for common viruses in healthy populations, particularly pediatric patients. The positive and negative predictive values for bacterial and fungal infections remain unclear. It cannot identify many pathogens that commonly affect neurosurgical and severely immunocompromised patients, and it does not include regional pathogens such as *Borrelia*, *Coccidioides*, and WNV. In addition, careful consideration must be given to pathogens associated with chronic infection and reactivation, such as herpesviruses, since detecting their presence does not always indicate pathogenesis. For example, Green et al⁷⁸ reviewed 15 patients with HHV-6 detected by FA-MEP, and determined that in only one patient was the clinical syndrome likely attributable to HHV-6; in two patients, HHV-6 was a possible culprit, while in the other 12 it was deemed unlikely to play a causative role. Finally, it remains unclear how to interpret the clinical significance of multiple potential pathogens in a single sample. For all of the above reasons, microbe-specific testing should be performed for confirmation in the setting of unexpected positive or negative results. It is incumbent upon clinical laboratories to provide assistance in reviewing results to avoid errors in clinical decision making.

Metagenomic Sequencing

General Approach and Use in Clinical Settings

Metagenomic sequencing is a relatively unbiased approach whose aim is to detect any pathogen using a single method. In this technique, all nucleic acid in a sample is sequenced simultaneously using a “shotgun” approach, and the resulting unbiased sequencing reads are depleted of human reads, then compared with reference databases to identify potential pathogens. Metagenomic sequencing libraries can be created directly from DNA to identify eukaryotes, bacteria, and DNA viruses. To detect RNA viruses, RNA is first reverse transcribed to complementary DNA using random primers, and metagenomic sequencing libraries are generated. These methods are depicted in ►Fig. 1 and described in greater detail in several recent reviews.^{105,106}

The past 5 years have seen an explosion in the use of metagenomic sequencing to detect and discover pathogens. Much of this work has been done in patients with CNS infection, since CSF is a sterile fluid lacking commensal microbes and there is relatively low background from host nucleic acid compared with other specimen types. Case reports have highlighted the capacity of metagenomic sequencing to detect pathogens belonging to different kingdoms. Examples include bacteria such as *Leptospira*¹⁰⁷ and *Brucella*,^{108,109} fungi such as *Candida tropicalis*,¹¹⁰ protozoa such as *Balamuthia*,¹¹¹ and viruses such as WNV¹¹² and hepatitis E virus.¹¹³ In addition to CSF, metagenomic sequencing has successfully detected pathogens in brain tissue,^{114,115} and in the bloodstream of a patient with a brain abscess due to *Scedosporium*.¹¹⁶ Although much of this work has been conducted in research settings, multiple case reports have demonstrated the clinical utility of rapid metagenomic sequencing.^{107,108,117,118}

Strengths and Limitations

Case reports have highlighted some of the remarkable strengths of metagenomic sequencing compared with standard testing. For pathogens that are commonly diagnosed by serology, metagenomic sequencing can offer a faster turnaround time¹¹⁸ and can achieve a diagnosis in patients in whom antibodies are unreliable, e.g., due to treatment with rituximab.¹¹⁹ For pathogens that can be detected by PCR, metagenomic sequencing offers advantages of high sensitivity and independence from pathogen-specific primers. Highlighting the benefit of using an unbiased approach, metagenomic sequencing has yielded unexpected findings in immunocompromised patients including coronavirus,¹²⁰ Cache Valley virus,¹²¹ and mumps vaccine-associated encephalitis.¹²² It can even identify novel pathogens.¹²³ When applied broadly, metagenomic sequencing can help reveal the importance of an underappreciated pathogen such as astrovirus.^{117,124–126} In addition to its direct diagnostic uses, metagenomic sequencing can also provide information about pathogen genomics and molecular epidemiology.^{122,127,128}

However, despite its many strengths, there are also limitations to metagenomic sequencing. Most importantly, it relies upon the presence of pathogen nucleic acid in the sample tested. As discussed, some CNS syndromes are believed to be caused not by the pathogen itself but by the resulting immune response, and metagenomic sequencing will not detect antibody-mediated processes. Some viruses replicate for a short duration in the CNS, e.g., WNV¹⁴; these will not be detectable by metagenomic sequencing routinely, but may be more easily detected in immunocompromised patients due to longer viral replication.¹¹² Adding to this challenge, in some cases, the utility of metagenomic sequencing cannot be predicted ahead of time. For example, enterovirus is readily detected by metagenomic sequencing in cases of meningitis.^{110,128,129} By contrast, in many recent patients with AFM, enterovirus has not been detected from CSF, but EV68 RNA has been detected in respiratory samples,¹³⁰ and EV68 is believed to be a causative pathogen for AFM.¹³¹

A second limitation to metagenomic sequencing is that its very high sensitivity leads to high background from microbes present on the patient's skin and from laboratory reagents. This leads to the need for careful interpretation of results and stringent use of negative controls.¹³² As an example, Salzberg et al¹¹⁵ detected sequencing reads from *Delftia* and *Elizabethkingia* in brain tissue samples from one patient with meningitis and another with a brain mass, respectively. The authors were appropriately skeptical of the causal role of these agents in causing the observed disease. A later study demonstrated that reads from *Delftia*, *Elizabethkingia*, and other organisms are present in many negative control samples, and suggested the use of a statistical scoring technique to distinguish signal from noise.¹³³

Standards for Clinical Metagenomic Sequencing

As metagenomic sequencing moves into clinical practice, it is becoming increasingly important to define its test characteristics, both through detailed use of negative controls to define specificity, as well as validation studies to define sensitivity. Validation for metagenomic sequencing is challenging because, unlike traditional tests, there are a multitude of possible outcomes, and it is not possible to validate every potential pathogen. It has therefore been proposed to validate a subset of organisms representing different general characteristics (e.g., viruses with different genome structures, or bacteria with different cell-wall types) using samples from patients with confirmed infection, as well as mock samples created both *in vitro* and *in silico*.¹³⁴ This approach has been described for CSF as well as respiratory samples, with report of overall sensitivity on the order of 70% and specificity >90%.¹³⁴ Using mock samples, the limit of detection for various organisms has been estimated to range between 10 to 100 copies/mL for viruses, on the order of 10 CFU/mL for bacteria, and between 0.01 and >100 CFU/mL for fungi.¹³⁴

Factors that can contribute to low sensitivity include a low level of pathogen nucleic acid, a high level of host nucleic acid as background, and a poor match between a particular pathogen's genome and the representatives included in the reference database. There is likely variability in these factors not only between types of pathogens (e.g., enterovirus versus WNV), but also between specific pathogens of the same type in different hosts (e.g., WNV in immunocompetent vs. immunocompromised patients). Factors that can contribute to low specificity include contamination from organisms on the patient's skin, microbe components present in reagents, or contamination from the laboratory environment or other samples included on the same sequencing run. False positives can also result from computational analysis, if there is poor specificity in the computational pipeline (e.g., classifying bacteria only to the level of genus) or misclassifications in the reference database.

Despite these challenges, there has recently been considerable success in establishing clinically validated pipelines for metagenomic sequencing for CNS¹³⁵ and respiratory¹³⁶ infections. This is also an active area of interest for industry, and it is likely that metagenomic sequencing will take on an increasingly important role in the diagnosis of infectious diseases.

Given the limitations described above, applying the results from metagenomic sequencing in clinical practice should involve careful interpretation in the context of a specific patient. For organisms not yet validated, confirmatory testing using a standard assay should be employed when possible. Currently, metagenomic sequencing is most often used as a "last resort" in patients in whom extensive other testing has been unrevealing. It is important to consider whether this would be more fruitful as a front-line test, where it could potentially replace standard PCR for common pathogens, detect rare pathogens without extensive additional workup, and perhaps have higher yield when applied to higher quality (i.e., fresh) specimens.

Future Outlook

The current approach to the diagnosis of CNS infections is complex, incorporating traditional tests such as culture, serology, and directed PCR, as well as newer broad-range tests such as multiplex PCR and metagenomic sequencing. Broad-range testing offers the exciting opportunity to detect many potential pathogens at once, and is expected to improve the overall rate of diagnosis for patients with CNS infection. However, the test characteristics and cost-benefit ratio of these methods are still being evaluated. Their routine use will require a cognitive shift among clinicians, lessening the emphasis on thinking of potential pathogens ahead of time, but increasing the need for careful interpretation of results.

Conflict of Interest

None.

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