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Clinical Utility of mNGS of CSF for Diagnosis of CNS Infections in Specific Patient Populations: Evidence Summary

Introduction

Acute central nervous system (CNS) infections such as meningitis and encephalitis remain undiagnosed in up to 50% of cases due to the limited scope, sensitivity, and speed of diagnostics currently used as standard of care[1,2]. Complex cases typically require numerous serial tests that are time-consuming, costly, and frequently inconclusive, leading to delays in treatment and poorer outcomes. Delve Detect CSF, a metagenomic next-generation sequencing (mNGS) laboratory-developed test created by UCSF and exclusively licensed and optimized by Delve Bio for rapid turnaround and high throughput, substantially expands pathogen detection and provides fast, reliable diagnosis of CNS infections directly from a single sample of cerebrospinal fluid (CSF).

Use Cases: When to Consider mNGS Testing in CNS Infections

The Delve Detect mNGS test analyzes all microbial genetic material in a CSF sample, enabling comprehensive and unbiased pathogen detection and substantially higher diagnostic yield than conventional methods[3,4]. Results are typically available within 2 days of sample receipt, facilitating faster diagnosis of meningitis, encephalitis, and meningoencephalitis, as well as earlier targeted treatment and improved outcomes. These advantages make it particularly valuable for a number of challenging clinical scenarios, as highlighted in multiple publications including a large-scale analysis of nearly 5000 CSF samples over 7 years of testing published in *Nature Medicine*[3]. Here, we highlight clinical scenarios where mNGS testing has demonstrated substantial clinical impact.

1. Expedited Diagnosis in High-Risk Patients

Critically-Ill Patients: For patients with severe or rapidly progressing CNS infections, timely pathogen identification is urgent to prevent irreversible neurological damage or death. Critically-ill patients often present with multiple overlapping clinical issues and non-specific symptoms, making targeted testing challenging and necessitating rapid, broad diagnostic evaluations with considerable resource utilization, including extensive testing and prolonged hospital stays. Delve Detect's turnaround time of 2 days is faster than culture-based methods, many serologic tests, and send-out PCR tests, with the added benefit of unbiased detection. In the *Nature Medicine* study, mNGS detected infections from pathogens known to cause severe or rapidly deteriorating CNS disease, such as Herpes simplex virus 1, *Cryptococcus neoformans*, and *Mycobacterium tuberculosis*, enabling targeted therapy and reducing reliance on broad-spectrum treatments with significant toxicities. By consolidating the diagnostic process into a single, rapid, and comprehensive test, mNGS improves clinical decision-making in critical time windows and helps optimize resource utilization.

Immunocompromised Patients with Opportunistic Infections: The overall diagnostic yield of mNGS was significantly higher in immunocompromised vs. immunocompetent patients (16.7% vs. 7.1%) in the *Nature Medicine* study cohort, with particularly high yields among HIV-positive individuals (41.7%), solid organ transplant recipients (21.4%), and hematopoietic stem cell transplant recipients (17.8%). This enhanced sensitivity highlights mNGS's critical role in identifying infections in populations susceptible to opportunistic pathogens, such as *Cryptococcus neoformans*, *Toxoplasma gondii*, and *Coccidioides* species, which are often identified late or excluded from conventional diagnostic workup. The test also identified invasive mold infections like *Aspergillus* and *Fusarium* species as well as viral infections like JC virus, which have high morbidity and mortality, particularly when diagnosis is delayed. mNGS is a crucial tool enabling early, targeted intervention in this diagnostically challenging and highly vulnerable group.

Pediatric Patients with Limited CSF Availability: Diagnosing CNS infections in pediatric patients is uniquely challenging due to limited CSF sample availability and the tendency for neonates and infants to present with vague or non-specific symptoms such as irritability, lethargy, poor feeding, or fever. Clinical workup can involve extensive testing, consuming significant CSF volumes and leading to repeat lumbar punctures. mNGS has proven especially useful in identifying atypical pathogens, such as Enterovirus, Herpes simplex virus, and *Toxoplasma gondii* in children with non-specific symptoms[3,4]. By consolidating diagnostic efforts, mNGS supports timely, targeted treatment while minimizing patient risk and addressing the challenges unique to pediatric populations.

2. Unclear or Atypical Clinical Presentations

Suspected Infections with Atypical or Fastidious Pathogens: Many CNS pathogens, including *Mycobacterium tuberculosis*, *Tropheryma whipplei*, and *Leptospira* species, are difficult or impossible to culture. CSF mNGS testing identified these and other pathogens that typically require specialized incubation or extended culture times, making it a valuable tool for diagnosing fastidious organisms. Additionally, the test identified 24 bacterial species, several fungal pathogens (including *Coccidioides* species and *Cryptococcus gattii*), and rare viral infections such as Powassan virus that are challenging to detect using standard methods and typically require specialized antibody testing that may only available through state or national public health labs[5]. By enabling broad, unbiased pathogen detection in a single assay, mNGS simplifies the diagnostic process, reducing delays and eliminating the need for multiple targeted tests. This breadth of detection highlights the critical role of mNGS in diagnosing infections caused by atypical pathogens, many of which are likely underdiagnosed or underreported.

High Clinical Suspicion of Infection Despite Negative Conventional Testing: Even when culture, PCR, or serology yield negative results, clinical suspicion for CNS infection remains high in patients with fever, altered mental status, flu-like symptoms, rash, and elevated white blood cell count or inflammatory markers. This is particularly true in those who have received prior antimicrobial therapy or have a suggestive exposure history. In the *Nature Medicine* study, mNGS testing identified 48 infections that were negative by all other methods, including cases of encephalitis caused by arboviruses such as West Nile virus and Powassan virus, which are challenging to diagnose and not included in traditional panels[5]. This underscores the utility of mNGS as an important testing option for patients with a high index of suspicion for an unidentified CNS infection.

3. Expanding Detection of Emerging, Environmental, and Re-Emerging Pathogens

Emerging or Unusual Pathogens in Patients With Complex Exposure Histories: mNGS enables the detection of diverse pathogens in cases with potential exposures to emerging or unusual organisms, including international travel, animal contact, contaminated food and water, insect or vector bites, and residence in endemic areas. These scenarios are becoming more frequent as climate change expands the geographic range of vector-borne and environmental infections. In the *Nature Medicine* study, mNGS identified waterborne infections like *Leptospira* species, parasitic infections such as *Angiostrongylus cantonensis*, and amoebic infections like *Balamuthia mandrillaris* and *Naegleria fowleri*. Notably, it also identified Potosi virus, a novel mosquito-borne arbovirus not previously reported in human infection, as well as Lone Star virus, another tick-associated virus of uncertain pathogenicity, in patients with fatal CNS disease[6]. Case reports further highlight the value of mNGS in diagnosing CNS infections with complex clinical histories. For example, mNGS identified longstanding *Brucella* meningitis in a patient subsequently determined to have a relevant animal exposure, demonstrating its ability to detect rare zoonotic infections not initially suspected[7]. mNGS also identified St. Louis encephalitis virus, a mosquito-borne flavivirus re-emerging in the southwestern US after more than a decade of inactivity, as the cause of fatal meningoencephalitis in an immunocompromised patient[8]. The pathogen had not been considered in the differential diagnosis and was only identified through mNGS after extensive traditional testing was negative. These examples underscore mNGS's utility in diagnosing infections tied to complex or evolving exposures and its growing role in addressing emerging infectious disease threats.

Supporting Outbreak Detection and Public Health Investigations: mNGS has shown utility in public health efforts, including investigations of outbreaks, hospital-acquired infections, and transmission events. For instance, mNGS helped identify *Fusarium solani* as the cause of a widespread international meningitis outbreak linked to epidural anesthesia, enabling targeted public health interventions such as larger-scale testing efforts and clinic closures[9]. Similarly, the detection of vaccine-derived yellow fever virus in an organ transplant recipient prompted a CDC-led investigation that identified additional transmission events from the organ donor[10]. mNGS testing also supports genomic surveillance by identifying specific pathogen strains and tracing transmission patterns in case clusters or hospital-acquired infections[11,12]. By uncovering rare pathogens, linking cases to environmental sources, and enabling strain-level differentiation, mNGS enhances outbreak detection, infection control, and epidemic preparedness.

4. Optimizing Treatment Decisions and Avoiding Unnecessary Therapies

Informing Decisions to De-escalate Antimicrobials or Initiate Immunosuppressive Therapy: Negative mNGS results can support clinical decision-making by increasing confidence that an active infection is unlikely, helping differentiate infectious from non-infectious causes of neuroinflammation, such as autoimmune encephalitis. This can allow clinicians to discontinue unnecessary empiric antimicrobial therapy, reduce further diagnostic testing, and proceed with alternative treatments such as corticosteroids or biologics when appropriate. In one reported case of CNS lymphoma and encephalitis, mNGS identified Epstein-Barr virus (EBV) as the sole pathogen without evidence of bacterial or fungal coinfection[4]. This finding supported the decision to discontinue empiric antimicrobial coverage and initiate chemotherapy and immunosuppressive agents without delay. With a reported negative predictive value of 92.3% in CNS infections[3], mNGS can help guide the safe de-escalation of antimicrobial use and shift diagnostic focus when

an infectious cause becomes less likely, ultimately reducing patient risk and resource use as well as expediting appropriate care.

Diagnosing Localized Brain Infections with Atypical Presentation: Localized brain infections can present with symptoms that do not clearly align with classic meningitis or encephalitis, such as chronic headaches, nausea/vomiting, focal neurologic signs, seizures, or vague non-specific complaints, complicating diagnosis and leading to delays. These infections are often challenging to pinpoint with conventional methods due to their ambiguous presentations and reliance on specialized or invasive testing, including CT-guided brain biopsy. mNGS can detect pathogens associated with these focal infections directly from CSF, including *Toxoplasma gondii*, *Taenia solium*, *Brucella*, *Coccidioides*, and *Cryptococcus* species, enabling earlier diagnosis and treatment while potentially avoiding invasive procedures and the associated patient risks and costs[3,7].

Conclusion

Delve Detect, a UCSF-developed mNGS test for CSF, represents a powerful advancement in diagnosing CNS infections by providing broad, unbiased pathogen detection with faster turnaround times and higher sensitivity than traditional methods. Its clinical utility spans critically ill, immunocompromised, and pediatric patients as well as those with atypical pathogens, emerging or environmental exposures, and localized brain infections, where challenges such as non-specific symptoms, diagnostic complexity, and low sample volumes often hinder timely and accurate diagnosis. Beyond individual care, mNGS supports public health efforts by detecting outbreaks and emerging pathogens. Integrating mNGS into clinical practice can help bridge diagnostic gaps, reduce invasive procedures, and ensure timely, targeted treatment, improving outcomes across a diverse range of patient populations.

References

1. Glaser CA, Gilliam S, Schnurr D, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000. *Clin Infect Dis*. 2003;36(6):731-742.
2. Granerod J, Tam CC, Crowcroft NS, Davies NWS, Borchert M, Thomas SL. Challenge of the unknown. A systematic review of acute encephalitis in non-outbreak situations. *Neurology*. 2010;75(10):924-932.
3. Benoit P, Brazer N, de Lorenzi-Tognon M, et al. Seven-year performance of a clinical metagenomic next-generation sequencing test for diagnosis of central nervous system infections. *Nat Med*. Published online November 12, 2024. doi:10.1038/s41591-024-03275-1
4. Wilson MR, Sample HA, Zorn KC, et al. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. *N Engl J Med*. 2019;380(24):2327-2340.
5. Piantadosi A, Kanjilal S. Diagnostic approach for arboviral infections in the United States. *J Clin Microbiol*. 2020;58(12). doi:10.1128/JCM.01926-19
6. Chiu CY, Godasi RR, Hughes HR, et al. Two human cases of fatal meningoencephalitis associated with Potosi and Lone Star virus infections, United States, 2020-2023. *Emerg Infect Dis*. 2025;31(2):215-221.
7. Mongkolrattanothai K, Naccache SN, Bender JM, et al. Neurobrucellosis: Unexpected answer from metagenomic next-generation sequencing. *J Pediatric Infect Dis Soc*. Published online January 6, 2017:iw066.
8. Chiu CY, Coffey LL, Murkey J, et al. Diagnosis of fatal human case of st. Louis encephalitis virus infection by metagenomic sequencing, California, 2016. *Emerg Infect Dis*. 2017;23(10):1964-1968.
9. Smith DJ, Gold JAW, Chiller T, et al. Update on outbreak of fungal meningitis among US residents who received epidural anesthesia at two clinics in Matamoros, Mexico. *Clin Infect Dis*. 2024;78(6):1554-1558.
10. Gould CV, Free RJ, Bhatnagar J, et al. Transmission of yellow fever vaccine virus through blood transfusion and organ transplantation in the USA in 2021: report of an investigation. *Lancet Microbe*. 2023;4(9):e711-e721.
11. Fridey JL, Stramer SL, Nambiar A, et al. Sepsis from an apheresis platelet contaminated with Acinetobacter calcoaceticus/baumannii complex bacteria and Staphylococcus saprophyticus after pathogen reduction. *Transfusion*. 2020;60(9):1960-1969.
12. Crawford E, Kamm J, Miller S, et al. Investigating transfusion-related sepsis using culture-independent metagenomic sequencing. *Clin Infect Dis*. 2020;71(5):1179-1185.