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The Utility of Metagenomic Next-Generation Sequencing (mNGS) in the Diagnosis of Meningitis & Encephalitis in Pediatric Patients: Summary of Data and Case Examples

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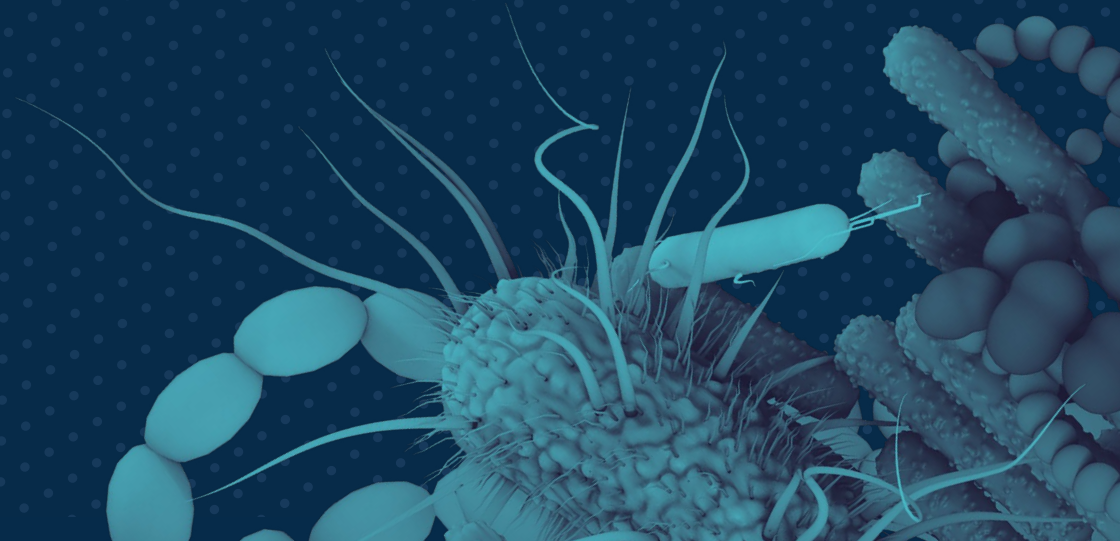

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Meningitis and Encephalitis in Pediatric Patients: Unknown etiology, extensive tests, limited precious sample, and urgent time pressures

Meningitis and encephalitis (ME) in children present a significant global health challenge, causing an estimated 190,000 deaths globally each year[15]. In the US alone, between 7,000 and 14,600 children under 18 are suspected of having ME each year[9].
A major hurdle in managing these cases is the high rate of unknown etiology, affecting up to 50% of pediatric patients.[3, 14, 19] This is often due to the non-specific symptoms children exhibit, which frequently lead to broad and potentially unnecessary antimicrobial use. The diagnostic process itself is often lengthy, demanding multiple lumbar punctures to collect sufficient cerebrospinal fluid (CSF) from children, procedures that may require sedation or anesthesia, further complicating and limiting diagnostic efforts. Such delays and the absence of a definitive diagnosis can compromise effective treatment, increase the risk of long-term neurological problems, and impose substantial burdens on families.

Percent of Pediatric ME cases with unknown etiology

Average Hospitalization Costs for Pediatric Patients with Suspected ME [11]

Based on a retrospective study of >6600 Patients across the US, 2011–2014

	Infants	Children
Mean hospitalization cost per patient	$12,800	$11,119
Mean hospitalization cost per patient for those with an unknown etiology	$41,397	$17,629

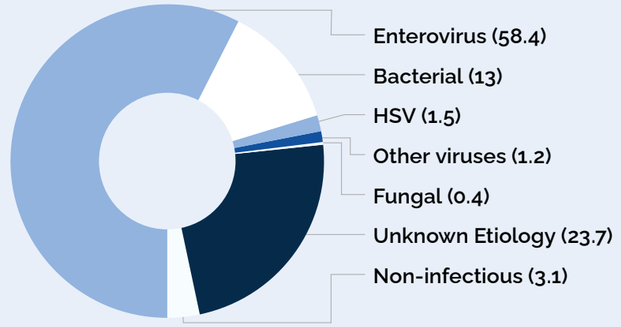
"Total visit cost increased with delayed LP procedure, ICU stay, and if the etiology was viral (other than enterovirus or arbovirus) or bacterial. Higher diagnostic and treatment costs were associated with delayed LP procedure, etiologic agent, and ICU stay."[11]

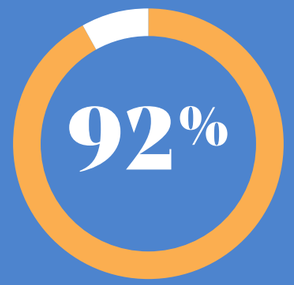
CNS infections in pediatric ME patients

Meningitis and encephalitis cases can stem from both infectious (viruses, bacteria, fungi, parasites) and non-infectious causes (neurologic, immune-mediated, neoplastic, metabolic, toxicologic disorders). Overlapping and non-specific clinical symptoms contribute to a lengthy diagnostic workup. More than 60% of ME cases[16] are caused by central nervous system (CNS) infections.

Traditional diagnostic methods like cultures are often slow and have a limited scope, while molecular tests such as PCR, though faster, require clinicians to pre-emptively suspect a specific pathogen, thus limiting their utility. These limitations often result in delayed or missed diagnoses, contributing to prolonged hospital stays and escalating healthcare expenses. The challenge is compounded by the small CSF volumes typically obtained from pediatric lumbar punctures, which can restrict the number of tests performed or necessitate repeat procedures.

Etiology (%) of Meningitis and Encephalitis in Children and Infants in the United States 2011–2014 [20]





Pediatric patients receiving intravenous antibiotics while waiting for CSF cultures

4.3x

Increase in costs of care for cases with unknown etiology (from $6,500 to $28,000) [11]

27.7

Average number of diagnostic tests ordered per patient [21]

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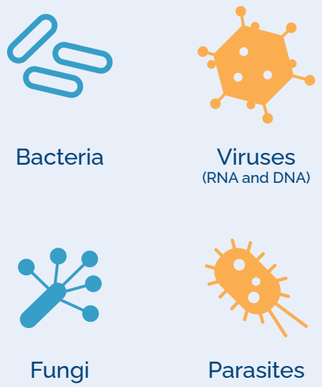
Delve Detect: comprehensive, agnostic, hypothesis-free detection of pathogens

Delve Detect is a metagenomic next-generation sequencing (mNGS) test that enables comprehensive, hypothesis-free identification of viruses (RNA and DNA), bacteria, fungi and parasites in a single sample. Delve Detect CSF's mNGS platform uses an unbiased approach to sequencing: sequencing DNA and RNA, including both cellular and cell-free nucleic acid in a single, 1 mL, sample of CSF. The process includes nucleic acid extraction, library preparation, sequencing, and bioinformatic analysis to identify the microorganisms present by mapping the detected sequences to an extensive microbial database of over 68,000 pathogens.

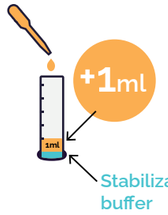
Delve Detect's results are rapid, crucial information that can quickly guide clinicians toward a definitive infectious diagnosis or help pivot the investigation toward a non-infectious etiology.

Detectable Microorganisms by Delve Detect

68,000



Turnaround Time
(after sample receipt)



Only **1 mL of CSF** sent in Delve Detect kit, **shipped at room temperature**

Includes **expert medical consultation** via Clinical Microbial Sequencing Boards

UCSF

Delve Bio is the new home of UCSF's CSF mNGS testing and exclusive licensee of UCSF's mNGS platform

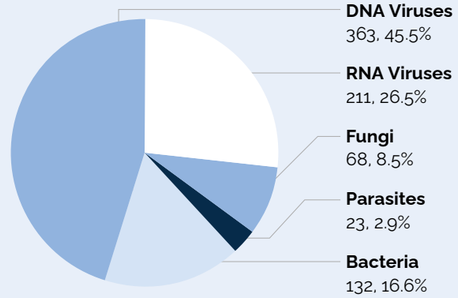
Delve Detect CSF for the diagnosis of suspected infectious meningitis and encephalitis

Delve Detect's results are rapid, crucial information that can quickly guide clinicians toward a definitive infectious diagnosis or help pivot the investigation toward a non-infectious etiology. Large scale analysis of nearly 5000 CSF samples over 7 years of testing at UCSF was published in Nature Medicine and demonstrated:

437
Unique Pathogens Detected

Broad Pathogen Detection

The pathogen diversity in CSF far exceeds the number of pathogens detected by conventional microbiological testing. In a 7 year analysis of the clinical use of CSF mNGS, over 400 unique pathogens were uncovered.[26]



22%
Additional Diagnostic Yield

mNGS has been shown to improve diagnostic yield in suspected central nervous system (CNS) infections, especially in cases where routine microbiological testing (such as culture and targeted PCR) fails to identify a pathogen.[26]

97%
Positive Predictive Value

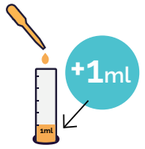
CSF mNGS exhibited positive predictive (PPV) and negative predictive (NPV) values of 97% and 92%, respectively, along with a specificity of 99.6% and accuracy of 93% for the diagnosis of CNS infections.[26]

86%
Sensitivity

The sensitivity of mNGS testing increased to 86% when comparing to diagnoses made by CSF direct detection (direct testing of the CSF to identify pathogens via methods like culture, antigen testing, and nucleic acid amplification testing [NAAT]) which justifies the use of diagnostic mNGS testing for hospitalized patients with suspected CNS infection.[26]

Key Advantages of Delve Detect for the Diagnosis of Pediatric ME

Low Sample Volumes



Current diagnostic workflow for ME can involve multiple tests (culture, PCR, serology); requiring higher volumes of CSF, which are limited and difficult to collect from pediatric patients. Available CSF volume can restrict how many tests can be performed, making it challenging to run all necessary tests, increasing pressure to choose the right test, and sometimes requiring repeat lumbar punctures.

Delve Detect CSF offers comprehensive pathogen detection with only 1 mL of CSF required.

Broad Differential

Pediatric ME can be caused by a wide array of infectious agents, including bacteria, viruses, fungi, and parasites, as well as noninfectious etiologies, which may lead to extended diagnosis and treatment timelines. Children often present with overlapping or nonspecific clinical features, making it difficult to narrow down the potential causative pathogen and guide targeted testing in a timely manner. Conventional microbiological tests are limited to pre-specified targets based on clinical suspicion, which can lead to missed diagnoses.

Delve Detect CSF leverages a database of over 68,000 pathogens, detected within a 48-hour turnaround time.

Broad, Costly, Empiric Therapy

Without definitive pathogen identification, children are often treated with multiple antibiotics, antivirals, or antifungals to cover all possible organisms. While this approach is necessary to avoid missing treatable infections, it exposes patients to unnecessary drugs if the causative agent is not present. Many empiric therapies for CNS infections, particularly newer or broad-spectrum agents, carry significant costs and risks of toxicity (such as kidney, liver, or bone marrow suppression).

Results from CSF mNGS have been shown to lead to positive clinical impact in 38.4% of pediatric cases, including ruling out concomitant CNS infection in 22.7% and identifying new infectious diagnoses in 9.3%. [12]

Complex Diagnostic Dilemmas

mNGS is especially helpful when conventional microbiological testing is inconclusive or when patients have complex presentations with overlapping symptoms. It augments traditional diagnostic approaches in challenging or atypical cases, potentially reducing unnecessary treatments and directing focus to genuine causes.

Studies have demonstrated that CSF mNGS can serve as a less invasive and more comprehensive diagnostic alternative to traditional candidate-based approaches or brain biopsies, especially in chronic neurological syndromes with elusive infectious causes. [22]

The Evidence: mNGS in pediatric patients

mNGS is revolutionizing the diagnosis of pediatric ME. By broadly detecting viruses, bacteria, parasites, and fungi from a single sample, mNGS tests like Delve Detect streamline the diagnostic process in pediatric patients.

Meta-analysis/Review

29

Publications

"mNGS has enormous potential to assist clinicians with the diagnostically challenging conundrum of pediatric meningitis and encephalitis." [4]

A systematic review [4] evaluated 29 studies and case reports involving children with suspected CNS infections who underwent mNGS testing. mNGS identified infectious etiologies in cases where routine testing (cultures, PCR, serology) was negative or inconclusive, often uncovering unexpected, rare, or novel pathogens (such as *Balamuthia mandrillaris*, *Leptospira*, astrovirus, and others). mNGS findings were directly responsible for changes in clinical management, including the initiation of targeted antimicrobial therapy and discontinuation of unnecessary empiric treatments. In some instances, mNGS results allowed clinicians to confidently pursue alternative (e.g., autoimmune) diagnoses when infection was excluded.

Clinical Impact

38.4%

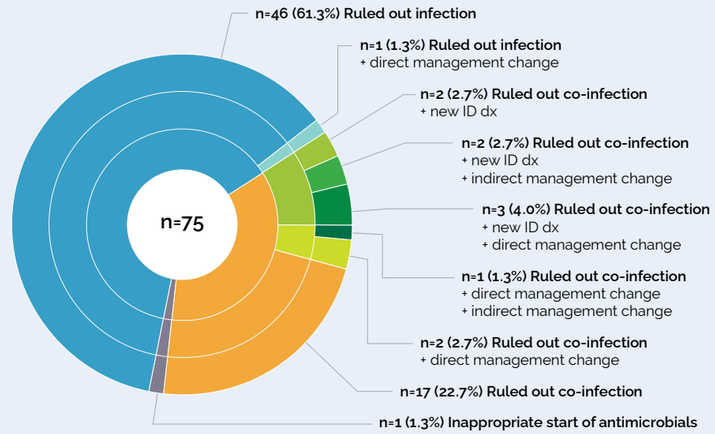
Pediatric cases with positive clinical impact

A retrospective evaluation specifically focused on a cohort of 193 pediatric patients tested using CSF mNGS at UCSF [12] found that CSF mNGS testing had a positive clinical impact in 38.4% of cases. This impact included ruling out concomitant CNS infection in 22.7% of these positive impact cases and identifying new infectious diagnoses in 9.3% of them.

38.4%
results impacted clinical care

61.3%
Ruled-out infection

97.1/92.3%
Positive predictive value/
Negative predictive value



Positivity Rate

28.6%

Positivity Rate in Pediatric Patients

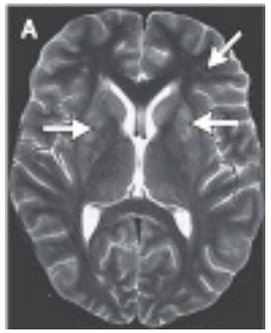
In a study of 70 children (from 221 screened) with CSF pleocytosis and suspected CNS infection at three U.S. pediatric hospitals enrolled over 12 months demonstrated a positivity rate of 28.6%. [2]

Case studies of Delve Detect CSF in pediatric patients

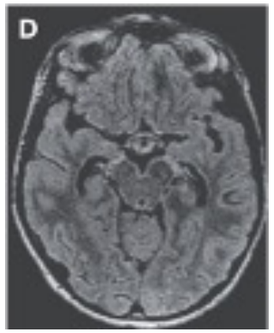
Case 1: When conventional testing fails

Actionable Diagnosis of Neuroleptospirosis by mNGS

A 14-year-old boy with severe combined immunodeficiency presented three times over 4 months with fever and headache. After the patient's condition continued to decline despite treatment for possible neurosarcoidosis and polyethylene glycol–modified adenosine deaminase (PEG-ADA) therapy, he developed new symptoms (e.g. worsening hydrocephalus, and status epilepticus) and required a medically induced coma. Around this time is when mNGS was performed on CSF and serum samples. Within 48 hours from sequencing, the analysis detected sequence reads corresponding to Leptospira infection in the CSF, directly leading to the decision to treat the patient immediately for neuroleptospirosis. The child was discharged 32 days later with a status close to his premorbid condition.[17]



MRI at 3rd hospitalization showed persistent hyperintensities in the basal ganglia and deep frontal white matter (where arrows point)



MRI post 3rd hospitalization shows near resolution of previously shown basilar meningitis

All Microbiology Testing Negative (shown in order of testing and re-testing for duplicates):

M. pneumoniae PCR
Histoplasma/Blastomyces antigen
Bartonella PCR
VZV/HHV-6/HHVB PCR
Borrelia burgdorferi PCR
Adenovirus/CMV/EBV PCR
HSV-1, 2 PCR
Enterovirus PCR
Cryptococcal antigen
Mycobacterial culture
West Nile Virus IgG/IgM and PCR
CEV/EEEV/WEEV/ SLEV IgG/IgM
Bacterial/fungal culture
Toxoplasma gondii PCR x2
Powassan virus PCR
Aspergillus antigen
JCV/BKV/HHV7 PCR
Viral culture (including mumps culture)
16S bacterial rRNA PCR
Bacterial culture
Adenovirus/CMV/EBV/VZV PCR
Enterovirus PCR
Epstein-Barr virus PCR
Varicella zoster virus PCR
EBV/ CMV PCR
Bacterial culture
Enterovirus PCR
Parvovirus B19/HHV7/ BKV/JCV PCR
Blastomyces/Histoplasma/Cryptococcus antigen
BKV PCR
Toxoplasma gondii PCR
Adenovirus/HTLV-1,II/ HIV/ HSV-1,2 PCR
16S bacterial rRNA PCR
Influenza A. B/RSV PCR
Respiratory Viral Panel (Luminex)
Mycoplasma pneumoniae PCR
Bacterial culture
Enterovirus PCR

In this case, UCSF's CSF mNGS offering specifically was used. Delve Bio is now the home of UCSF's CSF mNGS test.

Protracted illness & inconclusive diagnostics

Aug 2012 – Jul 2013
After swimming in freshwater in Puerto Rico, the patient developed recurrent symptoms including fever, headache, conjunctivitis, and thrombocytopenia. Despite multiple hospitalizations and comprehensive infectious disease evaluations, including CSF analysis and brain MRI, no cause was identified.

Critical deterioration

Aug 2013
During a third hospitalization, the patient's condition rapidly declined. The patient developed basilar leptomeningitis, hydrocephalus requiring an extraventricular drain, and status epilepticus. A brain biopsy showed granulomatous inflammation, but all conventional testing failed to identify the infection.

mNGS provides actionable diagnosis

Aug 21 – 23, 2013
Unbiased mNGS was performed on CSF and serum. Within 48 hours, 475 Leptospira reads were identified in the CSF. Based on this result, high-dose intravenous penicillin G treatment was promptly initiated, even before confirmatory testing was complete based on the clinical context of the patient and absence of any contamination indicators.

Rapid recovery post-targeted treatment

Late Aug – Sep 2013
Following targeted therapy, the patient showed rapid clinical improvement: seizures resolved, CSF normalized, and MRI findings improved. He was discharged to rehabilitation and returned home in near-baseline condition within 32 days of the NGS diagnosis.

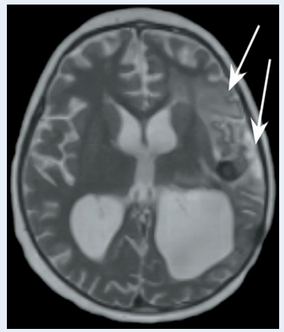
CDC confirmatory testing

Oct 2013 – Feb 2014
Months later, the CDC confirmed the diagnosis using a modified PCR on CSF (Jan 2014) and a novel IgM latex agglutination assay on serum (Feb 2014). These delays underscored the critical role NGS played in enabling a life-saving, timely diagnosis when standard tests failed.

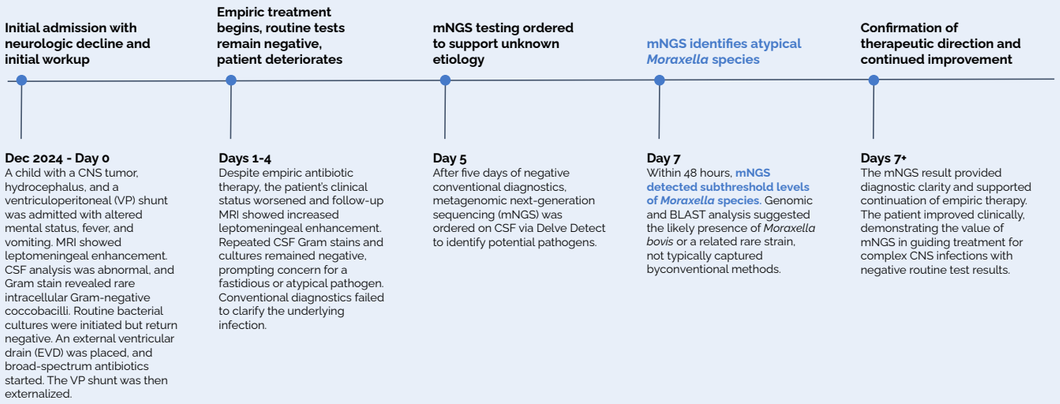
Case 2: mNGS provides confidence in treatment of shunt infection

Atypical Moraxella Causing a Culture-Negative Pediatric CNS Shunt Infection Despite Antibiotic Pre-treatment

A child under 10 years old with a history of a CNS tumor (astroblastoma), hydrocephalus, and a VP shunt, was admitted to the hospital with altered mental status, fever, and nausea/vomiting. The patient had a history of multiple and recent VP shunt revisions. An MRI showed extensive leptomeningeal enhancement. Initial CSF from the VP shunt was abnormal, showing increased WBC, high protein, and low glucose. A Gram stain showed rare intracellular Gram-neg coccobacilli, but routine CSF culture was negative. Despite meropenem for five days, the MRI findings worsened. The VP shunt was removed, but subsequent daily CSF Gram stains and cultures remained negative. At that time, Delve Detect was sent and identified a *Moraxella* species, potentially the first reported case of *M. bovis*/novel *Moraxella* CNS infection. The mNGS result provided clarity and confidence to the treating teams, allowing the patient to continue on meropenem, for the Gram-negative infection.[29]



MRI with extensive leptomeningeal enhancement, pointed out by arrows



Initial admission with neurologic decline and initial workup

Dec 2024 – Day 0
A child with a CNS tumor, hydrocephalus, and a ventriculoperitoneal (VP) shunt was admitted with altered mental status, fever, and vomiting. MRI showed leptomeningeal enhancement. CSF analysis was abnormal, and Gram stain revealed rare intracellular Gram-negative coccobacilli. Routine bacterial cultures were initiated but return negative. An external ventricular drain (EVD) was placed, and broad-spectrum antibiotics started. The VP shunt was then externalized.

Empiric treatment begins, routine tests remain negative, patient deteriorates

Days 1–4
Despite empiric antibiotic therapy, the patient's clinical status worsened and follow-up MRI showed increased leptomeningeal enhancement. Repeated CSF Gram stains and cultures remained negative, prompting concern for a fastidious or atypical pathogen. Conventional diagnostics failed to clarify the underlying infection.

mNGS testing ordered to support unknown etiology

Day 5
After five days of negative conventional diagnostics, metagenomic next-generation sequencing (mNGS) was ordered on CSF via Delve Detect to identify potential pathogens.

mNGS identifies atypical *Moraxella* species

Day 7
Within 48 hours, mNGS detected subthreshold levels of *Moraxella* species. Genomic and BLAST analysis suggested the likely presence of *Moraxella bovis* or a related rare strain, not typically captured by conventional methods.

Confirmation of therapeutic direction and continued improvement

Days 7+
The mNGS result provided diagnostic clarity and supported continuation of empiric therapy. The patient improved clinically, demonstrating the value of mNGS in guiding treatment for complex CNS infections with negative routine test results.

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Clinical Utility of Delve Detect CSF

Atypical Presentations

Atypical presentations of CNS infections often lack classic symptoms like fever, neck stiffness, or altered mental status. Patients may show vague or unusual signs such as persistent headache, subtle cognitive changes, or focal neurological deficits. This makes diagnosis challenging, as symptoms can mimic other conditions or be masked by co-existing illnesses, particularly in young children, immunocompromised individuals, or the elderly. Such nonspecific or unexpected presentations heighten the risk of delayed diagnosis and inappropriate treatment, underscoring the importance of comprehensive and unbiased mNGS testing.

> Murkey et al.[23] presented a lung transplant recipient who developed meningoencephalitis. The patient's atypical presentation, coupled with negative results from conventional testing, made diagnosis particularly difficult. CSF mNGS identified Hepatitis E virus (HEV) — the first reported case of HEV infection likely acquired through lung transplantation.

Suspicion of Infection Despite Negative Testing

mNGS plays a vital role in cases where conventional diagnostic tests return negative, and suspicion for infection remains high. Unlike targeted tests that rely on prior assumptions about the causative pathogen, mNGS provides unbiased and comprehensive detection of a broad spectrum of microbes, including bacteria, viruses, fungi, and parasites in a single assay. This approach is particularly useful for identifying rare, novel, or fastidious organisms that standard cultures or PCR may miss, especially after empirical antimicrobial treatment has begun.

> "Neurobrucellosis: Unexpected Answer From Metagenomic Next-Generation Sequencing" (2017) presents a case where >20 different conventional tests including blood cultures, 16S RNA, and fungal cultures, failed to diagnose the CNS infection. Nearly a year later, CSF mNGS identified *Brucella* as the causative pathogen, and toxic empiric therapies were de-escalated and the appropriate therapy of doxycycline and rifampin was initiated with full clinical resolution.[24]

De-escalate, Confirm, or Change Empiric Therapies

Metagenomic next-generation sequencing (mNGS) has been shown to significantly impact antimicrobial therapy by enabling clinicians to promptly discontinue unnecessary empiric treatments and initiate targeted therapy based on precise pathogen identification. This approach not only reduces exposure to broad-spectrum agents and their associated risks, but also improves patient outcomes by ensuring that therapy is tailored to the actual infectious cause.

> A 2021 multicenter prospective study by Wilson et al.[3] demonstrated that mNGS impacted clinical decision-making in 62% of patients, where it revealed an undetected infectious cause. In several cases, negative mNGS results reassured clinicians to discontinue empiric broad antimicrobials, expediting the transition to alternative therapy when infection was excluded as a cause of neuroinflammation.

Diagnostic Dilemma: Steroids or Antimicrobials?

A critical decision in managing ME is choosing between antimicrobial therapy and immunosuppressive treatment. A positive mNGS result supports the presence of an infectious agent and prompts targeted antimicrobial therapy, while a negative result helps exclude infection, enabling safe initiation of immunosuppressants or pursuit of alternative diagnoses such as malignancy.

> In a published case[25], a 15 yo patient initially received steroids and immunosuppressants because all conventional cultures and infectious work-ups were negative. By hospital day 5, her condition rapidly deteriorated with worsening headache, increasing weakness, and altered mental status. Repeat MRI on HD 5 demonstrated enlargement of hemorrhagic lesions; on HD 6, a biopsy showed significant tissue necrosis. The immunosuppressive therapy was ceased and broad spectrum antimicrobials were initiated; however the patient did not survive, underscoring the critical importance of accurate and timely pathogen identification in complex neurological syndromes.

Patient Populations for which to consider Delve Detect

Complex, Neurological Conditions

Many chronic CNS conditions are caused by rare, slow-growing, or previously unrecognized pathogens that are difficult to detect with routine cultures or targeted assays, especially after prolonged empiric treatment. mNGS provides unbiased, comprehensive pathogen detection in a single test, enabling identification of elusive infectious agents and distinguishing infectious from non-infectious causes when symptoms persist. This broad, rapid approach can clarify diagnoses, guide targeted therapy, and help avoid unnecessary treatments in patients with prolonged or unexplained neurological symptoms.

In a 2018 study, a set of patients with chronic meningitis who underwent extensive but inconclusive traditional microbiological testing were analyzed using CSF mNGS. Delve's cofounders were able to identify rare and unexpected pathogens that had not been detected by routine tests. These results show that mNGS offers timely interventions, and reducing unnecessary testing or invasive procedures in patients with chronic meningitis of unclear etiology. Amongst the pathogens found were *Taenia solium*, in 2 patients), a virus (HIV-1), and 4 fungi (*Cryptococcus neoformans*, *Aspergillus oryzae*, *Histoplasma capsulatum*, and *Candida dubliniensis*).[22]

Pediatric Patients

Symptoms of ME in children can be atypical and difficult to discern — clinicians often initiate very broad, costly, and untargeted antimicrobials while waiting for microbiological testing. mNGS can help reduce the overuse of antibiotics, which is a key driver of antimicrobial resistance and can expose children to unnecessary medication risks.

A 14-year-old boy with severe combined immunodeficiency presented three times to a medical facility over a period of 4 months with fever and headache that progressed to hydrocephalus and status epilepticus necessitating a medically induced coma. Diagnostic workup including brain biopsy was unrevealing. CSF mNGS by Delve's cofounders identified *Leptospirosis*. Targeted antimicrobial agents were administered, and the patient was discharged home after 32 days.[17]

Immunocompromised or Transplant Patients

Immunocompromised patients are at heightened risk for unusual, rare, or opportunistic infections that may not be detected by conventional testing. Immunocompromised patients often present with atypical symptoms, and traditional diagnostics can frequently miss or delay the identification of the causative pathogen.

A study from UCSF analyzed 7 years of CSF mNGS found that the positivity rate was over 2x higher in immunocompromised (16.7%) than immunocompetent (7.1%) patients. mNGS provided the first or only diagnosis in over 21% of cases[26].

Suspected Autoimmune Encephalitis

ME can have both infectious and noninfectious etiologies; differentiating between the two etiologies allows clinicians to avoid or discontinue unnecessary antimicrobial treatments, limit additional testing and procedures, and proceed with alternative therapies like corticosteroids or biologics. With a negative predictive value of 92.3% in CNS infections[26], Delve Detect assists with the clinical exclusion of infectious etiologies, reducing patient risk, and focusing care on non-infectious etiologies.

In a 2025, a study evaluating patients with encephalitis or similar CNS disorders of unknown cause showed mNGS helped to excluded infection in 14 cases (42.4%). In these patients, further comprehensive workup supported non-infectious diagnoses such as autoimmune or inflammatory conditions, neoplasia, and amyloid angiopathy. Clinicians were able to discontinue unnecessary antimicrobial therapy and safely initiate immunosuppressive treatments (e.g., corticosteroids, immunomodulators) when an infectious cause was ruled out.[27]

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