

METAGENOMICS 101

How Metagenomic Next Generation Sequencing is Revolutionizing How We Treat and Manage Infectious Diseases

Metagenomic Next-Generation Sequencing Speeds Diagnosis and Saves a Life

In 2014, the first case to demonstrate the ability of metagenomic next-generation sequencing (mNGS) to provide clinically actionable information for a patient with a diagnostically challenging infectious disease was described in the New England Journal of Medicine.

SEPTEMBER 2012

A 14-year-old boy with **severe combined immunodeficiency disease** had recently traveled abroad and swam in a freshwater lake. Upon return home, he was admitted to the hospital three times in four months with what began as fever and headache. Symptoms eventually progressed to fluid accumulation on the brain and epileptic seizures so severe he needed to be placed in a medically induced coma.

JULY 2013

An **extensive diagnostic workup of over 30 tests**, including a brain biopsy, found no cause for the boy's symptoms. Although the boy showed signs and symptoms of leptospirosis infection, conventional microbiological testing including standard blood serum and cerebrospinal fluid (CSF) tests for the bacteria were negative. As a last resort, the patient's doctors reached out to colleagues (now co-founders of Delve Bio) at UCSF - CSF from the patient was sent for **mNGS which identified DNA sequences consistent with infection caused by the bacteria Leptospira**.

AUGUST 2013

The boy immediately received the appropriate antibiotics and was discharged from the hospital a month later – almost as healthy as he had been before the infection.

Prior to NGS testing, 38 tests were run on the boy to find a microbial cause of his illness. PCR and serological testing performed later at the Centers for Disease Control and Prevention confirmed leptospirosis. Access to next-generation sequencing accelerated the boy's diagnosis and likely saved his life, making this **the first published case where mNGS was used to guide clinical treatment in a difficult-to-diagnose infectious disease**.

Burden of Infectious Disease on the healthcare system

Millions of clinical visits every year are due to infectious diseases. Diagnoses are frequently delayed or missed - this can lead to delays in appropriate treatment, thus raising the risk for severe complications and even death.

2.8 million

infections each year are caused by antimicrobial-resistant organisms, leading to 35,000+ deaths. [🔗](#)

48 million

estimated foodborne infections, 128,000 hospitalizations, and 3,000 deaths occur annually in the U.S. [🔗](#)

~1.7 million

patients contract health-care-associated infections (HAIs) during hospital care, and 99,000 die as a result. [🔗](#)

\$11.5 billion

is the annual cost of fungal diseases when accounting for direct medical expenses, lost productivity, and premature mortality. [🔗](#)

\$52.1 billion

is spent annually on inpatient care for sepsis, a condition responsible for approximately 1 in 3 hospital deaths. [🔗](#)



Infectious Diseases can be hard to diagnose

Clinical Presentation

- Overlapping symptoms (fever, leukocytosis, malaise) between infectious and non-infectious etiologies complicate recognition. [🔗](#)
- Immunocompromised hosts often present atypically, obscuring classic signs (e.g., negligible leukocytosis despite severe infection). [🔗](#)

Narrow Scope of Targeted Molecular Panels

- PCR assays detect only organisms included in the primer design; rare, novel, or unexpected agents can be missed. [🔗](#)
- Many commercially available multiplex PCR panels have <20 targets, limiting actionable utility. [🔗](#)

Limitations of Traditional Testing Methods Techniques

- Blood cultures miss ≥70% of bloodstream infections and take ≥24 h for preliminary results. [🔗](#)
- Many fastidious organisms do not grow in routine media and culture plates, leading to low sensitivity and false-negative cultures in up to 70% of chronic infections. [🔗](#)
- Prior antibiotic exposure suppresses growth, causing missed pathogens and delayed targeted therapy. [🔗](#)

Reliance on a clinical suspicion or hypothesis

- Survey of ID physicians showed that “diagnosis not considered” and “appropriate test not ordered” were top contributors to delays. [🔗](#)
- Early infectious disease consults can reduce mortality in *S. aureus* bacteremia and endocarditis, yet referrals are still delayed or omitted in many hospitals. [🔗](#)

Economic Impact of Delayed Diagnosis

Delayed diagnosis places a financial burden on the healthcare system as well as the patient.

Prolonged Hospitalization

Delayed diagnoses often lead to more severe disease progression, significantly prolonging hospital stays —13 days for fungal meningitis, 10 days for arboviral infections, and 7 days for bacterial meningitis. [🔗](#)

Complications and Readmissions

Delayed diagnoses lead to complications and readmissions. Up to 13% of ME patients are readmitted depending on etiology. A quarter may not be discharged home. Some go to nursing homes, rehab or hospice. [🔗](#)

Complicated Treatment

Advanced stages of infectious diseases typically require more complex, expensive treatments and interventions. Patients may receive an average 8-day course of antibiotics for bacterial ME. More than 40% of bacterial cases and nearly 60% of those of unknown etiology get antivirals as part of broad empiric therapy. Empiric and sometimes inappropriate, often overlapping, therapies raise costs and risks. [🔗](#)

Resource Strain

Surges in severe cases due to delayed identification can overwhelm healthcare resources, leading to shortages of beds, staff, and critical supplies, and necessitating emergency spending. [🔗](#)

Additional diagnostic testing

Unclear symptoms and delayed identification can lead to repeated or unnecessary diagnostic procedures, including more than 28 microbiological tests per patient, adding to healthcare expenditures. [🔗](#)



How conventional testing methods fall short

Conventional microbiological testing methods face several critical limitations that can hinder timely and accurate diagnosis, especially in complex or atypical cases.

DEPENDENCE ON A CLINICAL SUSPICION

Traditional methods require clinicians to suspect a specific pathogen and order the correct tests. If an organism is not considered, it will not be detected. Unusual, emerging, or rare pathogens are frequently overlooked, particularly in patients with atypical presentations or complex medical backgrounds.

LIMITED SCOPE

Most tests are designed to identify only a limited number of pathogens. For example, PCR panels for meningitis typically detect just 10 to 15 organisms, despite hundreds being capable of causing central nervous system (CNS) infections.

NEED FOR SERIAL AND STEPWISE TESTING ITERATIVE APPROACH

Because of their limited scope, conventional tests often necessitate a stepwise process—initial tests (such as Gram stain and culture) are followed by additional rounds of testing if results are negative. Testing algorithms can include 2nd, 3rd, or even 4th line microbiological tests in the quest for a diagnosis.

CASCADING DELAYS

If the initial test battery is inconclusive or the correct culture conditions are not chosen, further tests (second, third, or even fourth line) are ordered based on evolving clinical suspicion, which can substantially delay diagnosis.

OPERATIONAL BOTTLENECKS

Each round of testing adds time, increases resource use, and may require repeated sample collection.

Conventional, Microbiological Tests for Infections Fall Short

In clinical practice diagnostic testing is typically **narrow and targeted**, requiring clinicians to **choose or hypothesize** the likely pathogen(s) to be tested for. This serial method uses conventional, microbiological tests that only detect either the **pathogen directly** (i.e., culture or PCR) or the **host's immune response** (i.e., serology) to the pathogen. While effective for **common infections**, they have limited utility in atypical or diagnostically challenging cases.

Common microbiological methods

	CULTURE	PCR	GRAM STAIN	SEROLOGY
What it detects	Growth of live organism	Pathogen DNA or RNA	Bacteria that are gram negative/gram positive	Host antibodies/antigens
Primary use	Bacterial mostly, and if specialized testing available, fungal and viral infections	Primarily viruses, bacteria, fungi	Screen for the general presence of bacteria and guide initial empiric therapy	Infections where pathogen itself cannot be detected.
Advantages	Allows antimicrobial susceptibility testing	Sensitive and targeted; Detects some non-culturable organisms	Differentiates major bacterial groups	• Useful for diagnosis of past or recent infections • Non-invasive (blood sample)
Disadvantages	• Requires live organisms • Slow (24–72+ hours) • Labor-intensive • Misses non-culturable/fastidious organisms. • Fungal or viral cultures are typically insensitive and not commonly available	Limited to very specific targeted pathogens; a handful of targeted PCR tests, or small panels (10–20 targets) are widely available in labs; specialized molecular testing may only be available through reference labs.	Low sensitivity, nonspecific	• Variable sensitivity • May not distinguish current from past infection • Window period limits early detection • Cross-reactivity can cause false positives

Clinical Metagenomic Next Generation Sequencing: An Unbiased Approach to Infectious Disease Diagnosis

Metagenomic next-generation sequencing (mNGS) revolutionizes infectious disease diagnostics by enabling an agnostic, comprehensive search for any pathogen in a clinical sample.

Unbiased detection

Unlike conventional diagnostic tests that target specific, suspected pathogens, mNGS is agnostic, or hypothesis-free. mNGS casts a wide net, simultaneously detecting all the genetic material from all organisms that could be present in a sample, including bacteria, viruses, fungi, and parasites. This comprehensive and broad approach enables identification of both common, rare, and even emerging pathogens.

Culture-independent detection

mNGS identifies pathogens directly from patient samples without the need to grow them in the laboratory. This is especially valuable for fastidious or difficult-to-culture organisms (i.e., fungal or viral) that traditional methods might miss.



Short history of mNGS: from academia to bedside

mNGS has come a long way. From its use in a soil sample in 1998 to its deployment for surveilling Ebola and COVID strains from 2014 to the 2020s, it now has an increasingly important role in hospitals as a critical diagnostic tool for complex infections.

1998

The idea of **analyzing the metagenome of a soil sample** in order to identify novel organisms that might be useful for drug discovery was proposed. [🔗](#)

1990-2003

The **Human Genome Project** was conducted to determine the sequence of the human genome. This project drove technological advances, among which was next-generation sequencing (NGS).

2004

The **first commercial NGS platform** comes to the market. [🔗](#)

The first whole genome sequencing metagenomic study of microbial genomes conducted. [🔗](#)

2007-2010

The **Human Microbiome Project** is conducted using NGS platforms. It establishes the infrastructure and workflows that will be used in early infectious disease metagenomics. [🔗](#)

2008

First documented use of mNGS to diagnose an infectious disease, *Campylobacter jejuni* bacteria, in a stool sample from a Japanese man with severe acute gastroenteritis. [🔗](#)

2014

Researchers at the University of California San Francisco demonstrate **the value of mNGS** by diagnosing a critically ill boy with encephalitis with *Leptospira* just 48 hours after a CSF sample was taken. [🔗](#)

2014-2015

mNGS is used during **Ebola outbreaks** in Sierra Leone and Guinea. Samples are sequenced 18 to 48 hours after collection, enabling detection of distinct transmission chains, including sexual transmission months after recovery. [🔗](#) [🔗](#)

2015-2020

mNGS makes the transition from experimental to emerging routine clinical use for the diagnosis of complex infections.

2020-Present

mNGS transitions from research tool to frontline resource in outbreak management and antimicrobial resistance surveillance. **mNGS plays a critical role in the COVID-19 pandemic**, as it is used to identify new variants, trace their introduction into the population, identify transmission chains, and assess the impact of interventions. [🔗](#)

2022

Delve Bio becomes exclusive licensee of UCSF's mNGS technology platform and commercializes it for diagnosing infectious diseases.

Present

Delve Bio's mNGS platform, **Delve Detect**, is **now commercially available** for CSF and and backed by peer-reviewed data.

Why Getting the Right Sample Matters: Site of Infection

Infectious meningitis and encephalitis can involve a patient's cerebrospinal fluid (CSF) and/or brain. Analyzing these sites directly will result in the highest sensitivity and specificity, increased clinical utility of results by identifying the pathogen within the relevant site, and allows clinicians to target antimicrobial therapy, improving outcomes and reducing unnecessary broad-spectrum treatment.

Higher Sensitivity Maximizes Diagnostic Yield

Sampling CSF from patients with meningitis or encephalitis will more likely contain the higher concentration of causative pathogens than other potentially less invasive samples like plasma.

CNS infections can be caused by fastidious, rare, or novel organisms that may not be present in the bloodstream or may be missed by conventional testing. Direct sampling ensures these are not overlooked.

Higher Specificity Reduces False Positives

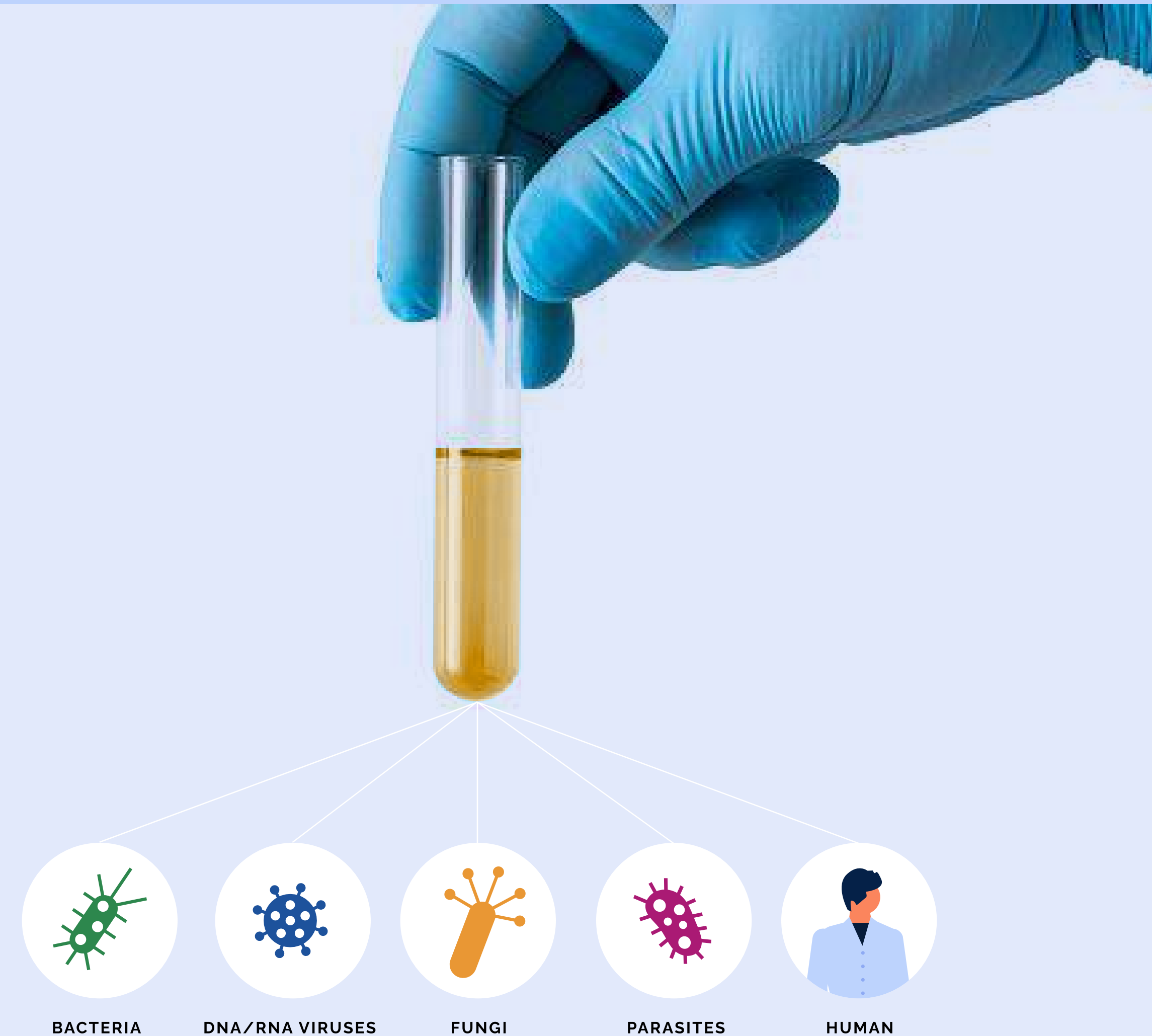
CSF has less background human DNA and fewer non-pathogenic microbes compared to blood or peripheral samples. This reduces the risk of detecting contaminants or organisms not responsible for the CNS disease.

CSF is typically relatively sterile with little to no pathogens present; pathogens detected in CSF are likely causative, providing actionable information for treatment.

CSF Samples are Accessible but Challenging

CSF is usually obtained through a lumbar puncture (spinal tap), by inserting a needle into the lower back to access the subarachnoid space. CSF samples can have a complex composition, presenting challenges for identifying pathogens. CSF samples are typically sterile, suggesting that any microbes found in a patient's CSF may be pathogenic - the underlying cause of the patient's symptoms. However, in practice, the CSF may contain other microbial nucleic acids, the result of contamination from reagents, lab equipment, or skin. Additionally, while generally acellular, host (human) DNA is present in the CSF in variable amounts from white blood cells, which generally increases in CSF in severe infections.

COMPONENT	TYPICAL RELATIVE ABUNDANCE	NOTES
Host nucleic acids (DNA/RNA)	>99%	Highly variable. Mostly from leukocytes and other cell types; dominates total nucleic acid content.
Host cells	5–10,000 cells/ μ L (pleocytosis)	Mostly white blood cells; normal CSF is nearly acellular.
Pathogen nucleic acids (bacterial, viral, fungal)	<0.01–0.1%	Varies widely by pathogen type, burden, and treatment status.
Normal microbial flora	~0% (sterile site)	CSF is normally sterile; any true microbe suggests pathology.
Environmental/contaminant DNA	<0.001–0.01%	Reagent, lab, or skin contamination (e.g., <i>Cutibacterium</i> , <i>Ralstonia</i>).



MNGS UNDER THE HOOD

From Sample to Raw Data

mNGS employs high-throughput sequencing technologies to directly identify microorganisms in a specimen through the detection and analysis of nucleic acids (DNA/RNA). The workflow can be divided into two parts: a wet-lab component and a dry-lab (computational) component.

Nucleic acid extraction

Lysis

mNGS analyzes microbial nucleic acids, which are located within cells or within pathogens present. These structures must first be lysed to release DNA/RNA for subsequent purification. Some microbial cells — such as fungi — are difficult to lyse. Extraction protocols must be optimized to recover nucleic acids from both easy-to-lyse and difficult-to-lyse pathogens.

Quality control

Use of control samples is essential to monitor for contamination during extraction.

Optimizing for RNA

Isolation and preparation of RNA can be challenging and require special consideration, including the use of stabilization buffers when storing and transporting the sample, and a separate extraction workflow, including a step that converts RNA to cDNA prior to sequencing.

Enrichment

While DNA from all cell types (human and microbial) within the sample are present, human DNA can often overwhelm the signal. Enrichment techniques are designed to increase the proportion of pathogen nucleic acids relative to host or background material, thereby improving sensitivity and efficiency in pathogen detection.

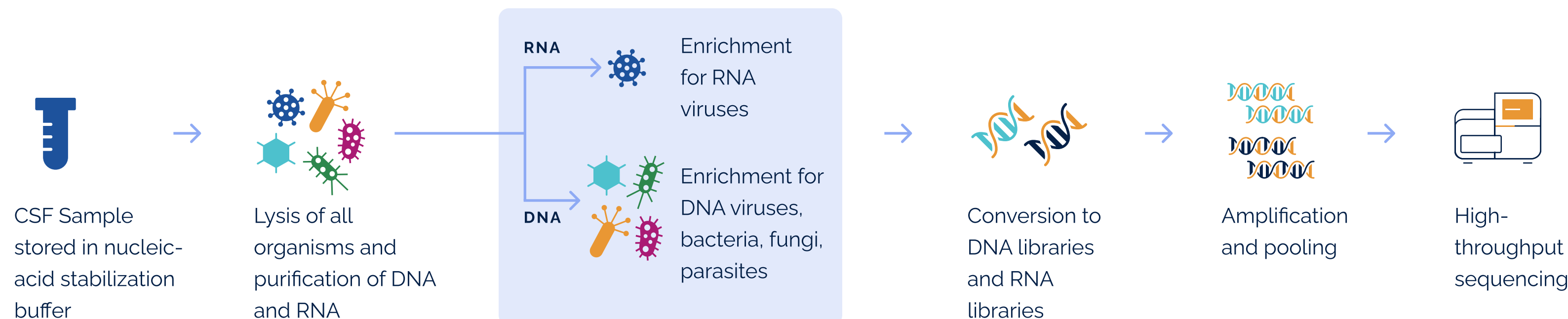
Library prep, amplification, and pooling

Next, all DNA (and RNA, which is converted to cDNA) in the sample is fragmented into small pieces, adding special adapters required for sequencing and using PCR amplification to increase the concentration of the DNA. This fragmentation of DNA into small pieces is necessary for the parallel, high-throughput sequencing methods referred to as next-generation sequencing (NGS).

Sequencing

Finally, the libraries are loaded onto a high-throughput sequencing instrument. The most common approach is sequencing by synthesis, where fluorescently labeled nucleotides are incorporated one by one, and the emitted signals are detected to determine the DNA sequence. The resulting, raw sequencing data (reads) are processed and analyzed using bioinformatics tools to reconstruct the sequence and identify the organisms from which they came.

Wet lab workflow



The RNA Advantage

While DNA is the relevant nucleic acid for yeast, parasites, fungi, bacteria, and some viruses, there are also important RNA viruses. RNA viruses may account for about 50% of CNS infections globally and are some of the the most common causes of viral encephalitis and meningitis. Enteroviruses are the most common in children, while arboviruses (e.g., West Nile Virus) are a growing concern in many parts of the United States. Including an analysis of RNA increases the cost of the test significantly. Nonetheless, it is a critical component for maximizing diagnostic yield by detecting RNA virus infections.

Optimizing the wet-lab workflow to improve accuracy and speed

The mNGS wet- lab process is nuanced and requires rigorous quality control and advanced automation to deliver clinical-grade results in a timely fashion. Few laboratories have optimized the mNGS workflow to deliver robust, accurate, and efficient analysis of samples required for consistent clinical use.

ESTIMATED GLOBAL PROPORTIONS OF CNS INFECTIONS BY PATHOGEN TYPE

25% DNA Viruses

(HSV, VZV, CMV, EBV, JC)

12% Fungi

(Cryptococcus, Candida, Aspergillus)

8% Parasites

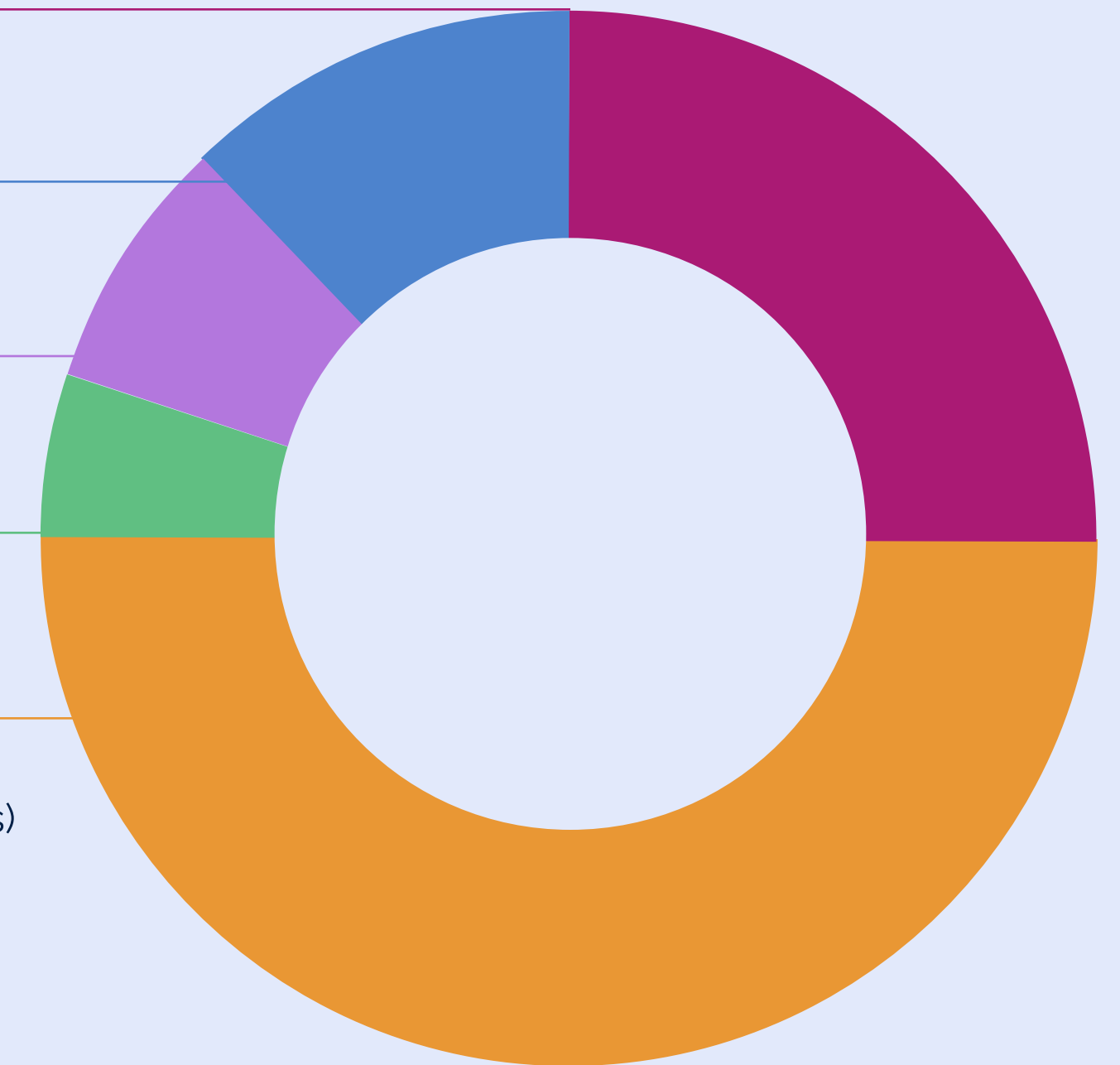
(Toxoplasma, Cysicercosis, Malaria)

5% Bacteria

(Strep pneumo, Neisseria, TB, Listeria)

50% RNA Viruses

(Enteroviruses, Arboviruses, Measles, Rabies)



mNGS Under the Hood: From Raw Sequence Data to Clinical Report

Delayed diagnosis places a financial burden on the healthcare system as well as the patient.

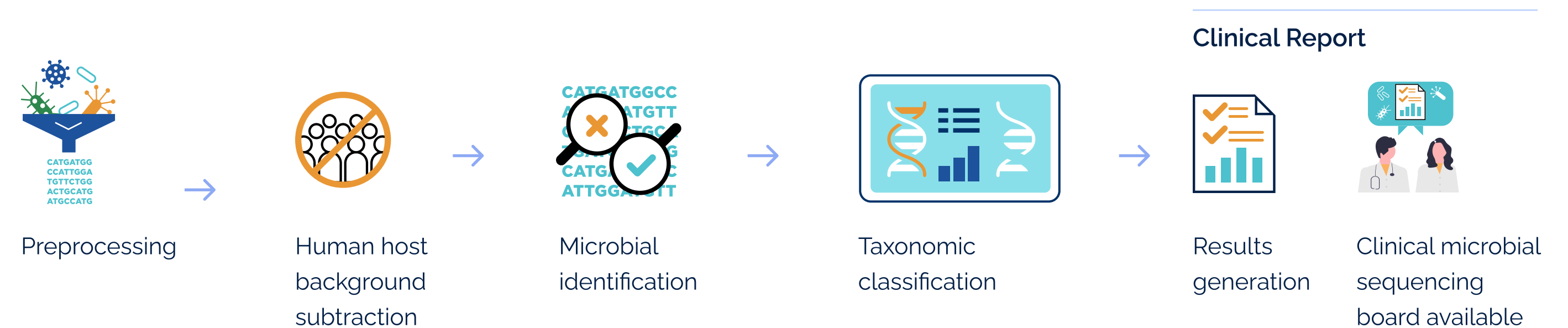
After the wet lab phase is completed — culminating in the generation of sequenced fragments of DNA — the dry lab phase begins. This computational phase of mNGS is essential for transforming raw data into meaningful clinical insights.

It requires a robust bioinformatics pipeline that integrates multiple data sources and analyzes large amounts of sequence data. This pipeline typically includes several key steps: pre-processing and quality control, removal of host (human) sequences, identification and classification of microbial sequences, and generation of clinical reports.

Advanced bioinformatics tools are used to integrate data from multiple sources, such as clinical metadata, reference genomes, and pathogen databases. The pipeline must be capable of handling large-scale datasets efficiently and accurately, ensuring that even low-abundance pathogens are detected without generating excessive false positives.



Delve's proprietary pipeline, **Delve Decide**, consists of a highly curated database, host filtering, and rapid identification to generate results within hours.



Raw data pre-processing and quality control

Before proceeding, the raw sequence data is pre-processed and cleaned - adapters are removed, and low-quality sequences are filtered out. The high-quality sequence data is passed on to the next step, which involves the identification of host and microbial DNA sequences using a curated and accurate database of organisms.

By rigorously cleaning and assessing the raw data, the risk of artifacts, misclassifications, and false positives in the final results is significantly reduced, ensuring that only the most accurate and meaningful data are used for clinical interpretation.

An Accurate and Comprehensive Database of Microbial Sequences is Crucial for mNGS to Work

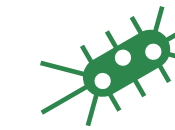
Database curation

Accurate identification of microorganisms relies on public repositories like the NCBI Nucleotide database, which contains genomic sequence data, including tens of thousands of species of microorganisms.

These repositories are inherently dynamic, frequently modified and updated, as new sequences are generated and deposited from laboratories around the globe. They can contain redundant, low-quality, or mis-annotated sequences due to their open and evolving nature. Moreover, they are not specific to organisms that have been or potentially could be linked to CNS infections.

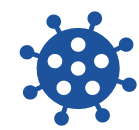
To maintain accuracy, it is essential to continually curate, update, and de-duplicate these datasets — removing spurious entries, incorporating new taxonomic insights, and optimizing for relevance to clinical diagnostics.

Classified microorganisms



Bacteria

>26,000 species



DNA and RNA Viruses

>10,000 species



Fungi

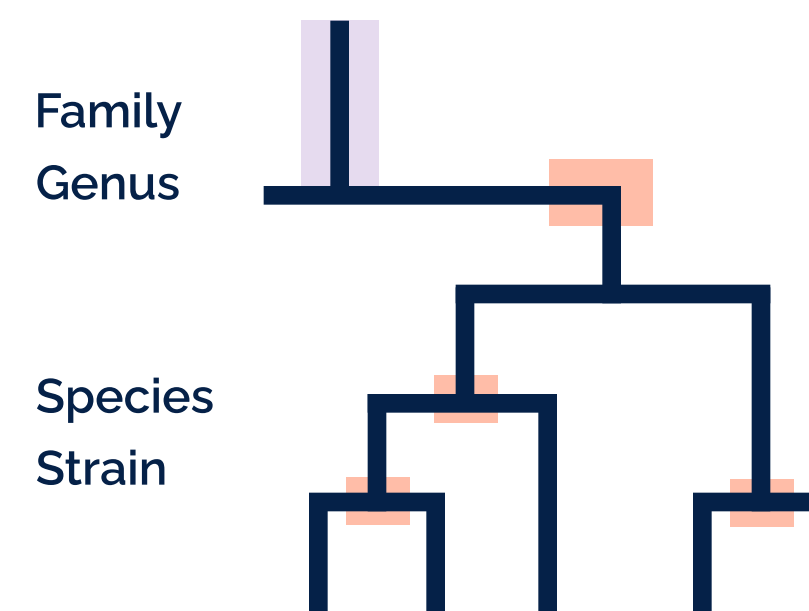
>16,000 species



Parasites

>15,000 species

MICROORGANISMS ARE FURTHER CLASSIFIED



Taxonomic classification and profiling

Depending on the degree of similarity to sequences found in the reference database, the sequenced fragments from the sample can be assigned to the family, genus, species, or even strain of the organism. Classification of an organism at the species level can inform treatment decisions, while strain-level information may be useful for tracking outbreaks.

Not All Steps in the mNGS Workflow Can Be Automated: The Role of Professional Judgement and Interpretation

Interpretation and reporting

The final output of the dry lab process is a set of concise, interpretable results. These results are reviewed and interpreted to highlight clinically relevant findings—such as the identification of pathogenic organisms, antimicrobial resistance genes, or unusual microbial profiles. The curated results are then compiled into a clinical report that provides physicians with clear, actionable insights to guide patient management.

Not all detected microorganisms in a sample are clinically relevant. Many may represent harmless commensals, environmental contaminants, or low-level background noise. Quantitative thresholds of microbial sequence abundance are established to distinguish true infections from low-level contamination. 

Moreover, filters can be applied to remove well-characterized commensal flora (e.g., anelloviruses, papillomaviruses) commonly found in healthy individuals.

The remaining microorganisms require manual review by professionals, such as pathologists and infectious disease specialists, who consider the clinical context of the patient along with published literature, databases of known pathogens, and clinical training to determine the likelihood of pathogenicity.

The final step is to present the findings through a clinical report, which may include not only the list of detected organisms, but also insights such as clinical interpretation and limitations.

 Delve Bio provides expert clinical and medical support through its Clinical Microbial Sequencing Board (CMSB) before, during and after test orders to ensure appropriate use and actionable clinical interpretation



How mNGS Compares with Conventional Microbiological Testing

Overview of conventional targeted diagnostic methods

There are several methods currently used to diagnose infections. These methods are usually **targeted**, meaning they test for specific, known microorganisms that are suspected of causing a person's symptoms.

Three of the methods detect the pathogen directly, while the fourth examines host response to the pathogen as an indirect measure of infection.

The ability to grow the live microorganism in **culture** is the gold standard for diagnosis. However, culture is best suited for non-fastidious bacteria that grow readily on standard or specialized media, and if they can be grown at all, it can take days to weeks.

PCR

detects genetic material (DNA/RNA) from a wide range of bacterial, viral, and fungal pathogens. Single-pathogen or multiplex panel tests are sensitive to low-titer infections but can also generate false positives. PCR offers high sensitivity and rapid turnaround, often providing results within a day.

Antigen testing

uses immunoassays to detect proteins from pathogens, but assays are available for only a small number of organisms. It is most useful in acute infections with high pathogen loads; results can be returned within minutes to hours.

Serology

is an indirect method of detecting pathogens by measuring antibodies produced by the host in response to infection. Antibodies detected in the blood or CSF can indicate infection, but this method has limited utility for acute infection, where antibody responses may take days to weeks to develop, and is best used for detecting chronic or past infections.

These conventional targeted methods remain foundational in clinical microbiology, each with distinct advantages, limitations, and applications depending on the suspected pathogen and clinical context.

	CULTURE	PCR	ANTIGEN TEST	SEROLOGY
What is it	Live pathogens grown on a specific medium and then identified by other methods	Amplification of microbial DNA/RNA using pathogen-specific primers	Detection of microbial antigens, typically surface proteins (Gram negative or Gram positive)	Detects host antibodies that bind to the pathogen
Single or multiplex assay	Only live organisms	Single, multiplex	Single	Single
Breadth	Primarily non-fastidious bacteria (fungal, viral cultures are less widely available)	Detection of a wide range of viral, bacterial, fungal, and parasitic infections	Limited to some viral, bacterial, and fungal infections	Limited to some viral, bacterial, fungal, and parasitic infections
Primary use	Often as the first line test to broadly assess the presence of pathogens. Also enables susceptibility testing	Typically used to detect specific viruses; specialty PCRs tests are less widely available for fungal or parasitic pathogens.	Most useful in acute infections with high pathogen load	Detection of recent or past infections. Limited use in early or acute infections.
Turnaround time	Days to weeks (depending on the microorganism)	Hours to days (as a sendout)	Minutes to hours	Hours to days
Sensitivity	Low-moderate. Some pathogens may be difficult or slow to grow, depending on the organism type and culture media used	Moderate-high. Can detect low pathogen burden, especially in the early stages of infection	Moderate, with high pathogen burden	Variable: Can be low in early stages of infection and falsely negative in immunocompromised patients
False-positive rate	Low to moderate	Low to moderate	Moderate, depending on organism cross-reactivity	Moderate to high: Due to prior exposure, vaccination, and cross-reactivity
Cost	Low	Moderate to high (\$50-200)	Low to moderate (\$10-100)	Low to moderate (\$10-100)

Delayed or Undiagnosed Meningitis and Encephalitis Can Have Lasting Consequences

Clinicians can identify a causative pathogen in only half of all meningitis and encephalitis (ME) cases [🔗](#). The large category of “culture-negative,” “aseptic,” or “unknown-cause” disease exacts a substantial and under-appreciated toll on patients and health systems.

ME can be caused by a broad range of etiologies - autoimmune, malignancy, and infection.

Delayed diagnosis and treatment of ME can cause **permanent brain damage, hearing loss, memory problems, learning disabilities, speech deficits**, and **motor function impairments**. Survivors of delayed or missed diagnosis often experience long-term or permanent disabilities, including **cognitive impairment, paralysis**, and **neuropsychiatric symptoms**.

Fewer than **50%**

Proportion of people with bacterial meningitis who exhibit all three key symptoms — fever, neck stiffness, and cognitive changes — that would lead a health care provider to suspect meningitis. For other patients, suspicion of meningitis may be dismissed.

13%

The increased risk of death each hour antibiotics are delayed treating bacterial meningitis.

\$650 million

annually is spent on encephalitis admissions of all causes in the United States, with an average cost of \$28,151 per admission; 60% of this spending involves undiagnosed cases. [🔗](#)

Test Spotlight: Multiplex PCR Panels

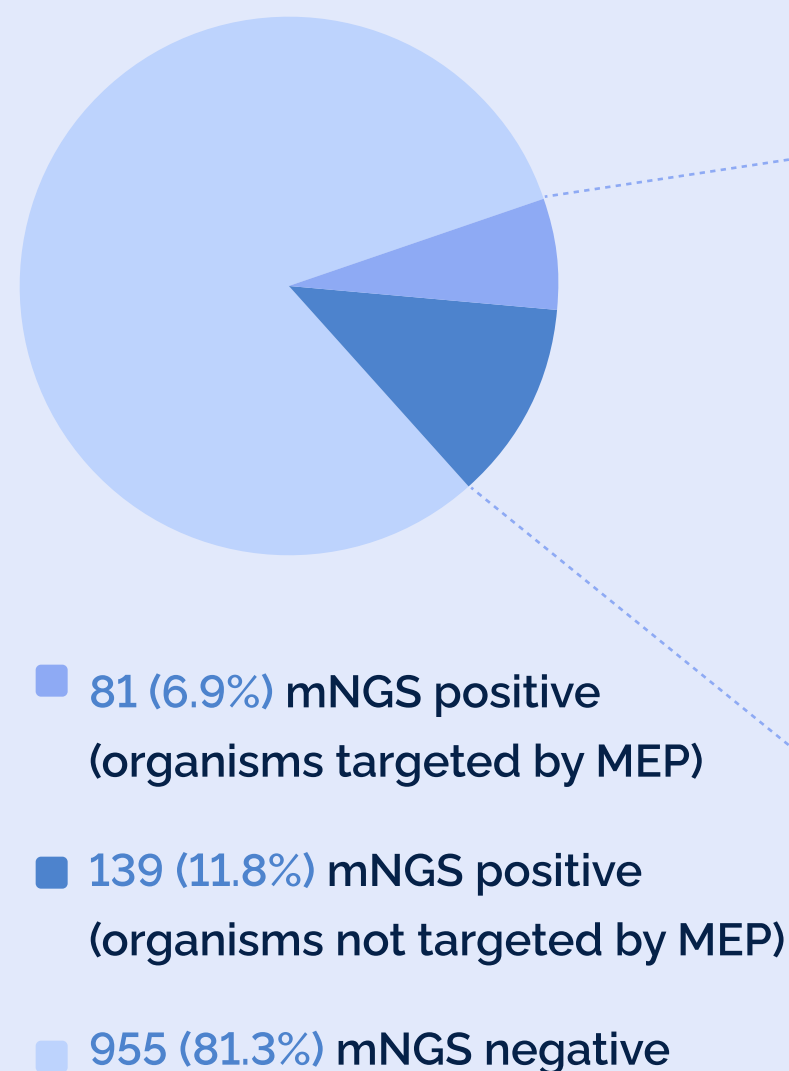
While most assays test for one or a few organisms at a time, PCR panels can be used for multiplex testing of several organisms simultaneously. While they can have a favorable turnaround time relative to mNGS, they are no match for the breadth of pathogens that can be detected with mNGS.

One such commercially available test is the BioFire FilmArray Meningitis/Encephalitis Panel (MEP), which targets 14 common pathogens, associated with meningitis and encephalitis.

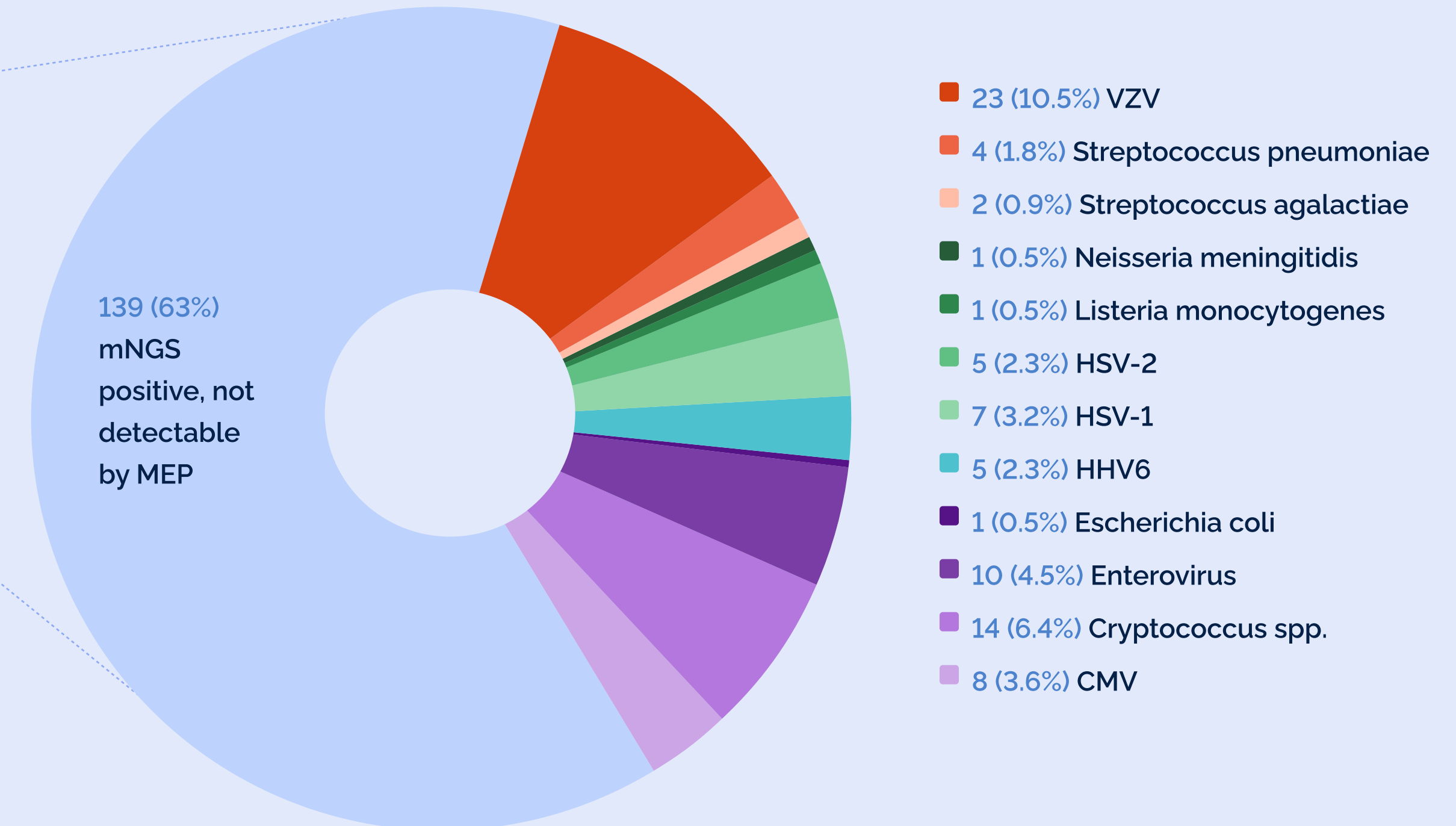
Real-World Performance

A study at UCSF evaluated the pathogen diversity from 1,164 CSF samples tested by mNGS. 220 samples had pathogens identified by mNGS, with only 6.9% of these potentially detectable in the MEP. Therefore, 63% of patients in this study had pathogens that could not be detected by MEP. In total, mNGS identified 437 distinct pathogen types, highlighting its unparalleled breadth compared to multiplex panels.

TOTAL OF 1164 CSF SAMPLES WERE TESTED BY mNGS



mNGS POSITIVE SAMPLES BY MEP TARGET STATUS





Test Spotlight: Multiplex PCR Panels

Turnaround Time and Practical Considerations

While multiplex PCR panels typically offer faster results than mNGS when performed in-house, this advantage can be lost if the test is sent to an external reference laboratory—a common practice in many hospitals.

When used as a sendout, panel-based testing can also increase costs and delay results, further diminishing the practical benefits of multiplex PCR panels in some settings.

	MULTIPLEX PCR PANELS (E.G., MEP)	mNGS
Number of pathogens detected	Limited (e.g., 14 for MEP)	1000s, depending on database
Turnaround time (in-house)	Fast (hours)	n/a
Turnaround time (send-out)	Multiple days	2-4 days
Cost	\$50-400	>\$2000

 Delve Detect can identify >68,000 organisms and returns results the next day after sample receipt.

Non-targeted, hypothesis-free microbial detection methods

For patients with atypical, severe, or diagnostically complex infections, hypothesis-free diagnostic methods are especially valuable. Unlike targeted tests, these approaches do not require clinicians to suspect a specific pathogen in advance—they can uncover a wide array of unexpected or novel organisms.

Non-targeted approaches include both mNGS and Universal PCR.

Universal PCR? (uPCR) (aka “16S rRNA”, “broad-range PCR”)

Universal PCR leverages genes that are highly conserved across a taxonomic group. The classic example is the 16S rRNA gene in bacteria, which contains conserved regions shared by most bacteria and variable regions that allow for species identification. Universal primers bind to the conserved regions, amplifying the 16S gene from a wide range of bacterial species, including those that are difficult to culture, in a single reaction. If a sample tests positive, sequencing the amplified product enables identification of the bacterial species present.

Primary Use: uPCR is primarily used in complex or culture-negative infections where it is most effective for detecting bacterial infections and is used less frequently for fungi or parasites. It is not suitable for viruses.

Clinical Availability: Only a few specialized laboratories, such as the University of Washington, have validated and implemented uPCR for routine clinical use with turnaround times ranging from days to weeks.

How Does uPCR Compare to mNGS?

mNGS and uPCR differ significantly in scope, flexibility, and clinical utility. The table below summarizes key distinctions:

	UNIVERSAL PCR (uPCR)	METAGENOMIC SEQUENCING (mNGS)
Target	Single gene (16S)	Entire genome of all organisms
Bacteria	Yes	Yes
Viruses	No	Yes
Fungi	Rarely (requires primers for 18S)	Yes
Parasites	No	Yes
Cost	\$500-2000 (with reflex sequencing)	>\$2,000
Turnaround Time	1–2 weeks	2-4 days
Samples Accepted	Wide variety, including brain tissue	CSF and plasma
Host DNA/RNA interference	Low	Moderate to high

mNGS: Not all tests are made equal

Karius and Delve Bio are commercial providers of clinical mNGS for infectious disease diagnostics, but they have important differences in technology, specimen type, pathogen coverage, and clinical applications:

Karius

Sample type: Blood (plasma) sample.

Analyte: microbial cell-free DNA (**mcfDNA**) from over 1,000 DNA pathogens, including bacteria, fungi, parasites, and DNA viruses.

Turnaround time: Results typically reported in 48 hours.

Clinical use cases include:

- Fever of unknown origin
- Suspected endocarditis
- Febrile neutropenia
- Sepsis
- Respiratory illness

Limitations: Lower sensitivity for localized infections (such as CNS) where cell-free DNA may not be present in sufficient amounts in the plasma. Does not detect RNA viruses.

Delve Bio

Sample type: Cerebrospinal fluid (directly sampling from the suspected location of infection).

Analyte: Microbial nucleic acid (DNA and RNA) from **both** whole organisms and cell-free fractions.

Turnaround time: Results available the next day after sample receipt.

Key technology: Built on technology developed at UCSF, Delve Detect analyzes both DNA and RNA in the sample, covering over 68,000 organisms.

Clinical use case: Suspected infectious meningitis and encephalitis

	KARIUS	DELVE BIO
Specimen	Blood (plasma)	CSF
Target	cfDNA	DNA & RNA
Organisms Detected	~1,000+	~68,000+
RNA Viruses	No	Yes
Turnaround	48 hours	48 hours
Clinical Sensitivity	60-80%	86%
Clinical Specificity	<50%	99% 

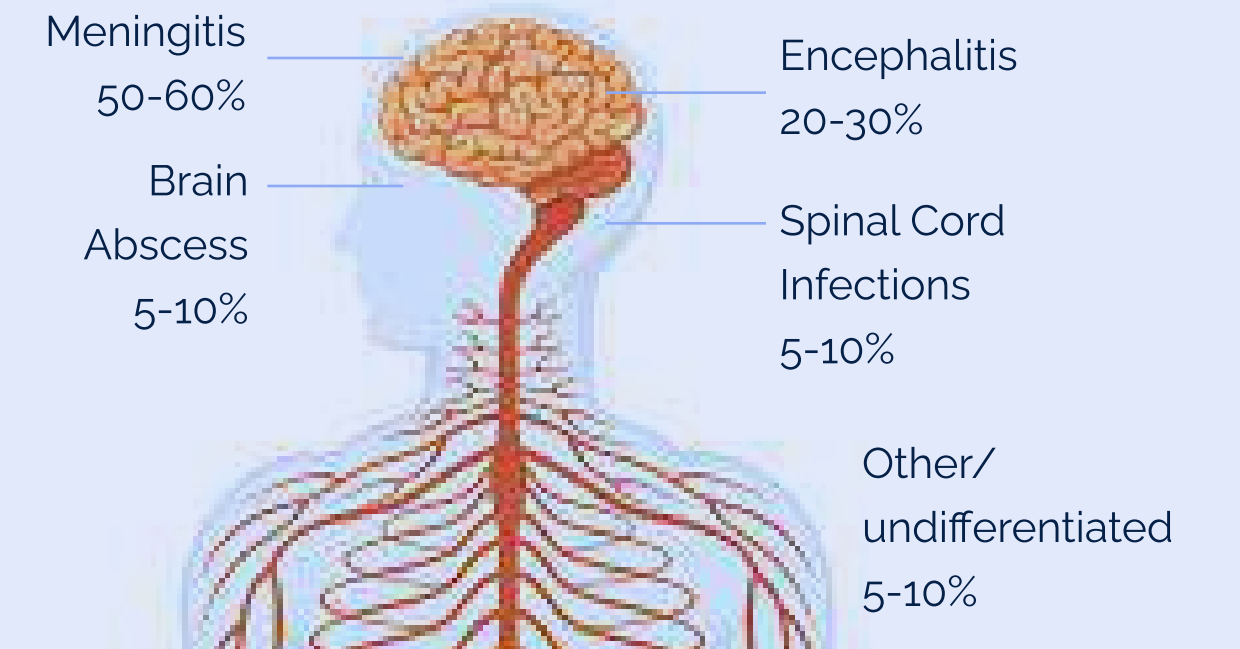
Burden of Meningitis and Encephalitis Diagnosis and Treatment

The Standard Diagnostic Workup of Meningitis and Encephalitis is Inefficient and Costly

Diagnosing meningitis or encephalitis often requires performing many laboratory tests (> 30 in some cases), significantly increasing healthcare costs and delaying the initiation of appropriate treatment.

The limited scope of conventional tests results in the need for multiple tests to be ordered. Some high-yield tests are ordered together at the outset, but if they come back negative, additional tests may be ordered based on the patient's clinical presentation, risk factors, and exposure history until a diagnosis is made.

MOST COMMON TYPES OF CNS INFECTIONS



This extensive testing process can be a major barrier to timely care, especially when rapid intervention is critical for patient outcomes.

INITIAL TESTS 0-1 WEEKS

WHITE BLOOD CELL (WBC) COUNT + DIFFERENTIAL
Neutrophils (bacterial), lymphocytes (viral/TB/fungal)
CSF CULTURE
Bacterial, fungal growth
CSF GLUCOSE & PROTEIN
↓ glucose ↑ protein in bacterial/fungal/TB

PCR TESTS AND/OR ME PANEL (MENINGOENCEPHALITIS)
Rapid viral/bacterial DNA detection (i.e., HSV, VZV)
GRAM STAIN
Rapid bacterial identification

IF INCONCLUSIVE AND INFECTION REMAINS ON THE DIFFERENTIAL

ADDITIONAL TESTS BASED ON SUSPICION 1-3 WEEKS

TB MENINGITIS
CSF AFB stain, TB PCR (e.g., Xpert MTB/RIF), culture
PCR
EBV, JCV, HSV, EV, etc
ADDITIONAL SPECIALTY TESTS
Additional test such as 16S, serologies, or targeted PCRs for less common pathogens

FUNGAL
India ink, cryptococcal antigen, fungal culture
SYPHILIS
CSF VDRL/FTA-ABS

PARASITES
Serologies (e.g., Toxoplasma IgG), eosinophils in CSF
ANTIGEN TESTS
Cryptococcal Ag

Burden of Meningitis and Encephalitis

27.7

Average number of diagnostic tests ordered to diagnose a CNS infection. [🔗](#)

13.3

Average length of stay for a patient with CNS infection [🔗](#)

13%

The rate at which the risk of death increases each hour antibiotics for bacterial meningitis are delayed. [🔗](#)

2 in 5

ME cases inappropriately treated with antiviral therapy. [🔗](#)

\$41,981

Average total inpatient visit cost of an infectious ME case [🔗](#)

40%

ME patients requiring admission to ICU [🔗](#)

40-60%

of cases remain undiagnosed, even after extensive diagnostic testing [🔗](#)

Confounding Symptoms and Diverse Etiologies of Meningitis and Encephalitis

CONFOUNDING SYMPTOMS

Meningitis and encephalitis often present with overlapping and nonspecific symptoms—such as headache, fever, neck stiffness, altered mental status, photophobia, and seizures—that can mimic a range of other neurological or systemic conditions. These confounding symptoms make it challenging to distinguish between different causes based solely on clinical presentation.

Given the broad range of possible infectious and non-infectious causes, as well as the overlap in clinical features, a comprehensive diagnostic approach is essential to identify the underlying etiology and guide appropriate management.

CLINICAL IMPLICATIONS

Given the diversity of potential causes and the urgency of treatment, the traditional approach—relying on multiple targeted assays—can delay diagnosis and compromise patient care. Broad, unbiased diagnostic methods are increasingly important for improving outcomes in patients with CNS infections.

75,000

Annual suspected cases of ME

60%

Proportion of ME Cases due to infectious agents

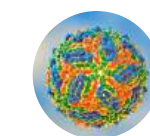
>400

Number of pathogens linked to ME

EXAMPLES OF INFECTIOUS ETIOLOGIES (CNS INFECTIONS)



Bacterial: *Streptococcus pneumoniae*, *Neisseria meningitis*, *Listeria monocytogenes*



Viral: Herpes simplex virus, enteroviruses, West Nile virus, varicella-zoster virus

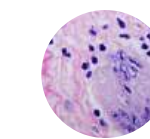


Fungal: *Cryptococcus neoformans*, *Coccidioides species*

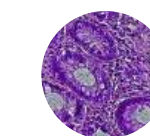


Parasitic: *Toxoplasma gondii*, *Naegleria fowleri*

NON-INFECTIOUS ETIOLOGIES



Autoimmune/Inflammatory: Autoimmune encephalitis (e.g., anti-NMDA receptor encephalitis), sarcoidosis, vasculitis



Neoplastic: Meningeal carcinomatosis, primary CNS lymphoma



Drug-Induced/Toxic: Adverse drug reactions, toxins



Post-Infectious: Acute disseminated encephalomyelitis (ADEM)

mNGS Identifies 22% More Pathogens (Missed by Conventional Diagnostics)

The sensitivity and specificity of mNGS are comparable to conventional testing methods, but the greatest diagnostic yield is seen when mNGS and conventional testing is used in conjunction with conventional testing. [🔗](#) [🔗](#)

Two representative landmark studies in the U.S. compared the performance of mNGS with conventional testing methods (PCR, culture, antigen, serology) among suspected CNS infections.

These two studies demonstrate that there is some degree of overlap in the ability of conventional testing and mNGS to detect pathogens associated with CNS infections. In approximately 22% of cases mNGS was the only test that identified the infectious agent.

naturemedicine

7-year Real World Evidence study

The largest study to date, researchers at UCSF carried out a seven-year retrospective study of using mNGS for diagnosing CNS infections in over 4800 samples, with over 20% of infections diagnosed only by mNGS. [🔗](#)



The NEW ENGLAND
JOURNAL of MEDICINE

PDAID study: Prospective Clinical Trial of CSF mNGS

This was the first large prospective study showing the diagnostic validity and utility of mNGS in CNS infections. Among 204 patients with suspected infectious disease, 28% were diagnosed as infectious; 22% of these were detected only mNGS and led to direct treatment changes in 54%. [🔗](#)

Strengths of mNGS

mNGS is able to identify additional infections that are missed by conventional diagnostic methods, potentially **increasing the diagnostic yield by >20%**.

Among infections found by mNGS alone, ~40-50% were missed by conventional tests because the **pathogen wasn't even considered** by the ordering physician, or there was **no test available** for the specific pathogen.

Additional false-negative conventional tests primarily resulted from **inability to culture** fungal or mycobacterial pathogens or effective **antimicrobial treatment** being administered prior to sample collection.

mNGS was also able to detect **novel/emerging organisms**, for example, Potosi virus, a bunyavirus not previously described in human infections and originally identified by screening of mosquito pools.

Limitations of mNGS

Most cases found by conventional methods alone were due to low titer or complete **absence of the pathogen in the CSF**. Instead, direct testing of infected tissue (brain tissue or abscess fluid) or serology testing was more effective at diagnosing the infection.

Clinical Impact of mNGS: Enabling Appropriate and Targeted Treatment

mNGS provides a powerful tool for solving diagnostically challenging CNS infections, especially when conventional methods fail. For patients, it can improve outcomes and spare unnecessary treatments. For health care systems, it enhances efficiency and precision in care, making it a valuable addition to the infectious disease diagnostic toolbox.

Results from the NEJM study (PDAID) found that for the diagnoses made by mNGS alone, over half of the diagnoses enabled appropriate and **targeted treatment**, including moving from broad- to narrow-spectrum antibody therapy.

CLINICAL IMPACT OF DIAGNOSIS OF MNGS (13)

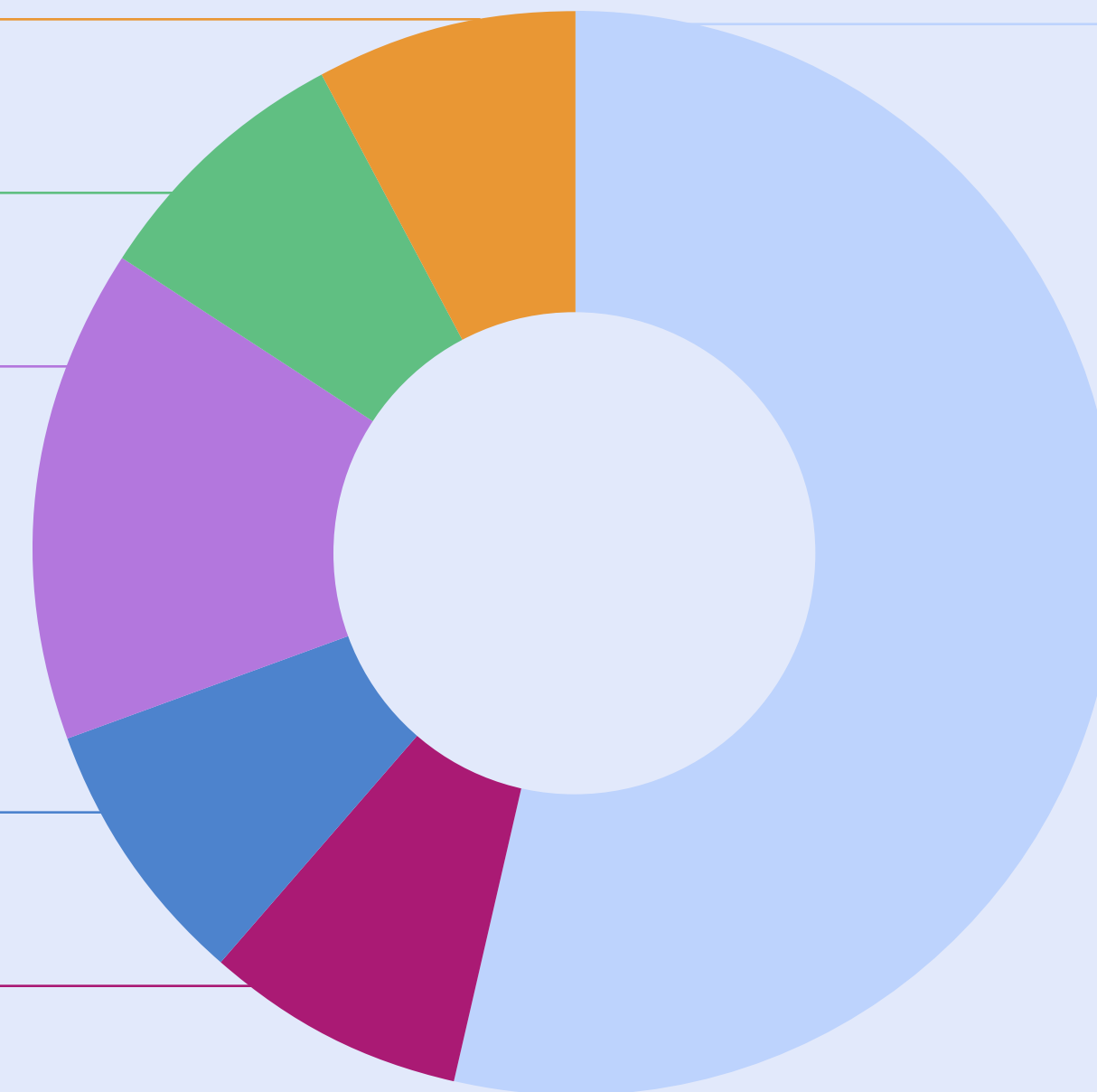
1 (8%) provided reassurance to patient surrogate (SLEV)

1 (8%) No effect. Clinical significance unclear (MW polyomavirus)

2 (15%) Had no effect because patient already discharged from hospital (enterovirus)

1 (8%) Supported clinical decisions to narrow coverage (neisseria)

1 (8%) Rule out coinfection; enabled patient to proceed with chemotherapy (EBV-associated lymphoma)



7 (54%) Enabled appropriate and targeted treatment

N. farcinica

long-term treatment with oral moxifloxacin and minocycline

Candida tropicalis

treatment with high-dose fluconazole and liposomal amphotericin B (started empirically for elevated 1,3-β-D-glucan level)

HEV

successful treatment with IV ribavirin after patient was readmitted with liver failure and consideration of liver transplantation

E. aerogenes

narrowing of antibiotic therapy to IV cefepime and oral trimethoprim-sulfamethoxazole

Enterococcus faecalis

narrowing of antibiotic therapy to IV vancomycin; discontinuation of meropenem

S. mitis

narrowing of antibiotic therapy to IV cefepime; continuation of antibiotics for 4 wk to treat CNS infection

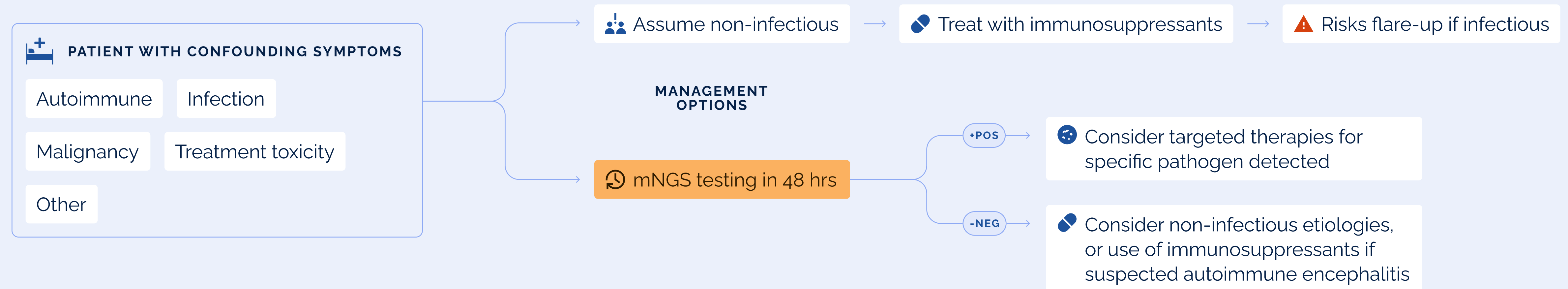
S. agalactiae

treatment with an additional 4 wk of therapy with IV ceftriaxone and vancomycin

mNGS: critical in the differential for complex ME cases

In addition to detecting pathogens, mNGS has utility, even with negative results, to help a provider assess that a patient has a low likelihood of infection. For example, in complex, undiagnosed cases of meningoencephalitis, the differential diagnosis often includes not only infection, but also autoimmune diseases that are

treated, often empirically, with steroids and other immunosuppressive agents. Confidently excluding infection would enable rapid deployment of immunosuppressants and ensure that a yet-to-be-determined infectious etiology does not further progress.

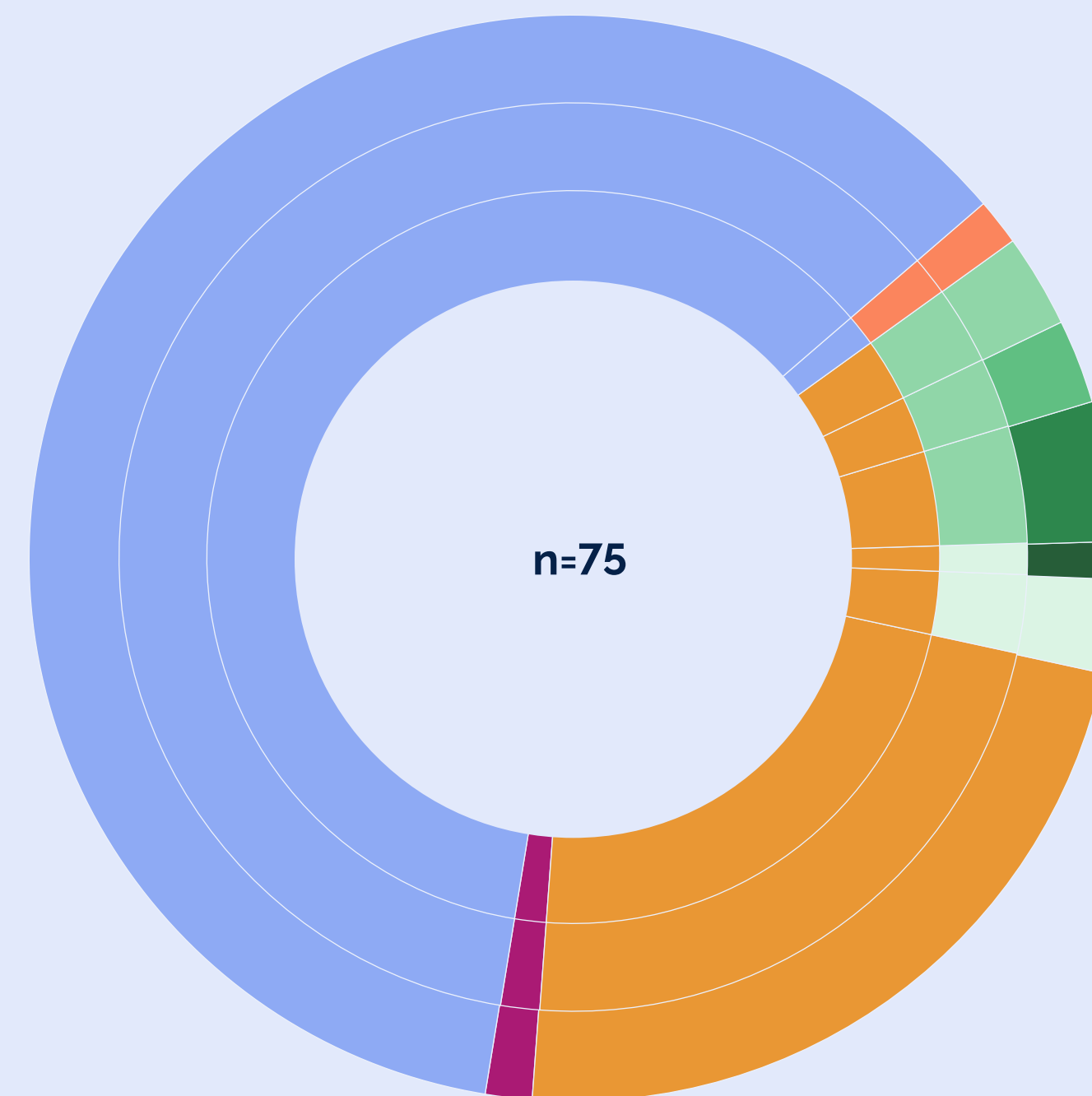


Clinical Utility of mNGS in Pediatric Patients

Results from a large cohort at UCSF found that diagnosis by mNGS had a meaningful clinical impact in 38% of cases, including exclusion of infections and leading to a direct treatment change.

In this study, the negative predictive value of CSF mNGS testing was 90.3% (vs 87% for all direct detection tests combined).

IMPACT OF MNGS IN A PEDIATRIC COHORT (N=193)



- n=46 (61.3%) Ruled out infection**
- n=1 (1.3%) Ruled out infection**
direct management change
- n=2 (2.7%) Ruled out co-infection**
new ID dx
- n=2 (2.7%) Ruled out co-infection**
new ID dx
indirect management change
- n=3 (4.0%) Ruled out co-infection**
new ID dx
direct management change
- n=1 (1.3%) Ruled out co-infection**
direct management change
indirect management change
- n=2 (2.7%) Ruled out co-infection**
direct management change
- n=17 (22.7%) Ruled out co-infection**
- n=1 (1.3%) Inappropriate start of antimicrobials**

38.4%

Cases with positive clinical impact

22.7%

Cases where co-infections were ruled out

10.7%

mNGS results led to a direct treatment change



Summary of clinical utility of mNGS

Targeted treatment

mNGS provides species-level identification and enables clinicians to tailor broad, empiric therapies that were prescribed initially in the absence of an etiology - knowing the pathogen can confirm which therapies should be used or not used, reducing use of broad-spectrum antibiotics.

Confidently exclude pathogens

Helps discontinue or de-escalate unnecessary broad-spectrum antibiotics/antivirals when no pathogen is detected, and more rapidly pursue noninfectious etiologies (i.e., autoimmune or oncology)

Shortened diagnostic journey

When facing a complex etiology, mNGS can offer a more rapid diagnosis, over the serial and inefficient testing paradigm with conventional testing.

Avoids invasive procedures

A definitive diagnosis may prevent the need for brain biopsy or repeat lumbar puncture in the quest for a diagnosis, infectious or not.



Is My Patient a Candidate for mNGS Testing?

There is a large body of evidence from prospective and retrospective cohort studies as well as real-world data, the following clinical scenarios may be considered for mNGS testing:

Pediatrics cases

In pediatrics, lumbar punctures often yield **very limited CSF volumes**, forcing clinicians to choose only a small number of tests. mNGS helps maximize the diagnostic yield from that first sample, reducing the need for repeat procedures and minimizing delays.

Immunocompromised patients

Solid organ and stem-cell transplant recipients, patients with hematologic malignancies, and those on intensive immunosuppression face an expanded universe of **opportunistic and atypical pathogens**. Conventional panels simply do not cover this diversity, and prior antimicrobial exposure often blunts culture yield.

Undiagnosed, microbiological negative cases

When conventional tests are negative, but neurologic symptoms continue or worsen, mNGS testing may provide answers that other tests cannot by detecting **viruses, parasites, bacteria, and fungi** in a single sample.

Complex unknown cases

There is a growing interest in using mNGS early when a rapid, comprehensive **exclusion of infection** would materially change management—for example, in patients with suspected autoimmune encephalitis where neurologists are weighing high-dose steroids, IVIG, or even biopsies.

When to Order an mNGS Test

The current workup for a CNS patient with suspected infectious etiology includes several rapid diagnostic tests that target a few specific, common organisms. Additional diagnostic tests may be ordered depending on the results of the initial tests and based on clinical suspicion.

1.

Comprehensive mNGS is used in conjunction with traditional methods

Rapid, highly accessible tests, such as CSF cell count, glucose/protein, and Gram stain, remain the first tests performed. These are inexpensive, fast, and answer critical questions quickly, and typically are performed in-house.

2.

Infection remains on the differential despite negative testing

When the differential is broad or the patient is particularly high risk, rather than ordering a dozen low-yield tests in series, clinicians can send one comprehensive assay that is capable of detecting RNA and DNA pathogens (bacteria, viruses, fungi, and parasites) from the same CSF aliquot.

3.

Atypical presentations

Patients especially those with overlapping encephalitis/myelitis, or those with prominent movement or psychiatric features where both infectious and autoimmune etiologies are plausible. In these cases, mNGS can identify a culprit pathogen or, equally important, support a more confident pivot to an autoimmune workup.



What mNGS Will Tell You That Other Tests Won't

mNGS delivers a level of diagnostic breadth and detail unmatched by conventional testing methods. Unlike targeted assays, which only detect specific, suspected pathogens, mNGS provides a comprehensive, unbiased analysis from a single sample and a single test. mNGS empowers clinicians with actionable information that extends far beyond the reach of conventional or targeted diagnostic methods, enabling faster, more accurate, and more comprehensive infectious disease diagnosis.

Unique Advantages of mNGS

Comprehensive Pathogen Detection

Simultaneously identifies viruses, bacteria, fungi, and parasites—regardless of whether they were initially suspected.

Unbiased Discovery

Detects pathogens that may have a non-textbook presentation, may not have been considered by the treating clinician, including rare, unexpected, or emerging organisms.

Identification of Novel Pathogens

Capable of uncovering new, previously undescribed infectious agents that would be missed by targeted tests.

Detection of Co-Infections

Reveals multiple infections in the same sample, providing a more complete picture of complex or atypical cases.

Superior Diagnostic Yield

Finds 22% more pathogens than conventional testing alone.

 Using Delve Detect has been shown to deliver 22% greater diagnostic yield, uncovering etiology in more patients.

CASE 1

In a dramatic example, mNGS saved a woman from having an unnecessary liver transplant

A 58-year-old woman with a suppressed immune system developed meningoencephalitis and decompensated liver disease shortly after a lung transplant. Admitted five years later with fever, headache, vomiting and neck stiffness, all microbiology testing returned negative results. Initial diagnosis included viral meningoencephalitis or tacrolimus toxicity. Using mNGS of her CNS, doctors identified Hepatitis E virus (HEV).

virus (HEV). This unbiased mNGS approach not only detected the uncommon pathogen but also provided clear evidence to guide clinical management, saving the patient from an unnecessary liver transplant. This rapid, precise diagnosis enabled clinicians to administer targeted antiviral therapy and stop unnecessary empiric treatments, significantly altering the patient's diagnostic and therapeutic journey.

2011

Lung transplant

tacrolimus, mycophenolate mofetil, prednisone, teriflunomide, trimethoprim sulfamethoxazole, acyclovir

2 WEEKS POST TRANSPLANT

Admission

Decompensated liver disease, encephalopathy, asterixis, ascites, and cirrhosis diagnosed by abdominal ultrasound and liver elastography

2013

Admission

Acute encephalopathy

2016

Admitted with severe headache

Fever of 38.3C, stable pancytopenia, elevated transaminases, negative for Hepatitis A,B, and C, brain MRI shows no new findings, cirrhotic liver

vancomycin, ceftazidime, acyclovir, voriconazole; tacrolimus was initially held but subsequently resumed

2016

Hepatitis E Virus-Associated

Follow up testing confirmed that the patient had acquired HEV during lung transplant. Patient was treated with ribavirin and low-dose diuretics. HEV virus RNA declined, transaminases normalized, and ascites improved. Identification and treatment of HEV prevented patient undergoing liver transplant.



58-YEAR-OLD WOMAN
MENINGOENCEPHALITIS

- Immunocompromised, post-lung transplantation, underlying multiple sclerosis
- Five years of anomia (word-finding difficulty) and slurred speech
- One year of recurrent episodes of dizziness and falls
- One month of lower extremity weakness

ALL MICROBIOLOGY TESTING NEGATIVE

Blood

CMV DNA quantitative PCR negative
Cryptococcal Ag negative
EBV PCR detected <10
HSV-1/2 PCR negative
Fungal culture negative
Bacterial culture negative
Toxoplasma gondii DNA PCR negative
MTB Quantiferon-Gold assay negative
Adenovirus DNA PCR negative
Parvovirus B19 DNA PCR negative
West Nile IgG / IgM negative
Rickettsia RMSF and typhus IgG / IgM negative
Varicella zoster DNA PCR negative
Coccidioides IgG and IgM EIA negative
Hepatitis A Ab total negative
Hepatitis A Ab IgM negative
Hepatitis A IgM negative
Hepatitis B core IgM negative
Hepatitis B surface antigen negative
Hepatitis C Antibody negative
HBV DNA PCR negative
Cryptococcal antigen negative
HIV 4th generation Ag/Ab negative
JCV Ab 2.31 >0.40 positive
RPR negative
Aspergillus antigen EIA negative
Coccidioides IgG/IgM EIA negative
Toxoplasma gondii DNA PCR negative

CSF

Cryptococcal Ag negative
Enterovirus PCR negative
Fungal culture negative
Bacterial Gram stain and culture negative
VZV PCR negative
HSV-1/2 PCR negative
CMV DNA quantitative PCR negative

Nasopharyngeal swab

Respiratory Virus Panel (RVP) PCR negative

MRI brain

Stable periventricular and subcortical and juxtacortical T2/FLAIR white matter intensities w/ T1 hypointensity

Initial abdominal ultrasound

Normal liver size, homogeneous in echogenicity. Normal spleen size. No ascites. Normally distended gallbladder containing sludge, without stones.

Peritoneal fluid

Bacterial gram stain and culture negative

Follow-up abdominal ultrasound

Cirrhotic appearing liver without focal lesion, patent hepatic vessels. Small volume ascites. Spleen upper normal in size.

Liver ultrasound elastography

shear wave liver stiffness
2.1 m/sec consistent with METAVIR score of F3-F4

Open Forum Infectious Diseases

BRIEF REPORT

Hepatitis E Virus–Associated
Meningoencephalitis in a Lung
Transplant Recipient Diagnosed by
Clinical Metagenomic Sequencing

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Chelsea L. Shannon,¹ Deepika Sirohi,¹ Hannah A. Sample,³
Michael R. Wilson,¹ Paul Vespa,¹ Romney M. Humphries,² Steve Miller,⁴
Jeffrey D. Klausner,¹ and Charles Y. Chiu,^{1,5}

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CASE 2

Multiple admissions, extensive testing, 3-month delayed diagnosis until mNGS

A 60-year-old woman with follicular lymphoma on immunosuppressants had multiple hospital admissions over several months with persistent fever and upper-respiratory symptoms. Despite extensive diagnostic testing, all were negative. Empiric antiviral and antimicrobial treatments were administered multiple times. After three hospitalizations and several negative workups, mNGS detected Enterovirus A,

leading to a rapid change in clinical management. Immunosuppressants (rituximab) were halted, intravenous immunoglobulin (IVIG) therapy was initiated, and experimental antiviral treatment (pocapavir) was started. mNGS provided clarity, averting unnecessary therapies and enabling targeted intervention.



60 YEAR OLD WOMAN
PERSISTENT FEVER AND UPPER RESPIRATORY SYMPTOMS

- Immunocompromised due to follicular lymphoma
- Taking rituximab
- CNS presentation

ALL MICROBIOLOGY TESTING NEGATIVE

HSV/VZV
Arbovirus
West Nile Virus
Crypto Ag
Toxoplasma PCR
Fungal PCR panel
Autoimmune encephalitis panel

OCTOBER 2024

Admission with fever, upper-respiratory symptoms

Diagnosed with COVID-19
Treated with remdesivir

OCTOBER

Re-admission with fever, upper-respiratory symptoms

Testing for several viral and fungal infections were negative
Treated with empiric antivirals antimicrobials

NOVEMBER

Re-admission with fever, upper-respiratory symptoms

Underwent third lumbar puncture and admitted to the ICU. Retesting for viral and fungal infections were negative

DECEMBER 4

Additional testing, including Delve Detect

Additional testing, including Delve Detect

DECEMBER 6, 4:30PM

Delve Detect identifies Enterovirus infection

Rituximab halted indefinitely, IVIG started immediately, experimental pocapavir started

🕒 36 hours

Delve Detect delivered results within the next day.

CASE 3

mNGS provides confidence in empiric treatment in critically ill patients with unknown etiology

A 10-year-old patient with a history of astroblastoma and a recent shunt revision exhibited clinical signs of CNS shunt infection. A positive cerebrospinal fluid (CSF) Gram stain suggested bacterial infection, but all culture results were negative, likely due to prior antibiotic use, or inappropriate growing medium. mNGS detected Moraxella DNA in the CSF sample consistent with the Gram stain morphology. The case

highlights the diagnostic limitations of traditional culture-based methods for CNS shunt-associated infections and demonstrates the clinical value of mNGS for culture-negative, device-associated infections. Phylogenetic analysis confirmed the Moraxella strain is related to a known species but is a distinct, potentially novel organism. Patient treated with broad-spectrum antibiotics and additional surgical options were considered.



10-YEAR OLD
CHILD
SHUNT-
ASSOCIATED
INFECTION

- History of astroblastoma
- Prior antibiotic treatment
- Recent shunt revision with clinical signs of infection

ALL MICROBIOLOGY
TESTING NEGATIVE

Gram staining: GN coccobacilli
Daily shunt fluid testing: Culture negative

DECEMBER 2024

Admission with fever, altered mental status

MRI with leptomeningeal enhancement

DAY 0

Shunt removal

Bacterial infection suspected, Gram-negative coccobacilli, culture negative

Treated with meropenem, a powerful, broad-spectrum antibiotic

Extraventricular drainage placed

Acute encephalopathy; daily shunt fluid testing, negative for culture

DAY 5

Delve Detect testing

While awaiting results, additional surgical procedures are considered as it is unclear if the broad spectrum antibiotics are adequate.

DAY 7

Delve Detect results identifies Moraxella DNA

mNGS confirms bacterial infection of a novel species related to Moraxella. Clinicians received expert consultation with Delve's Clinical Microbial Sequencing Board, who provided critical context for results.



Expert consultation with Delve's Clinical Microbial Sequencing Board provided critical context for results.

CASE 4

One Sample, One Test: mNGS spares children unnecessary procedures and treatment

An 11-year-old girl presented with headache, vomiting, and abnormal movements. Initial CSF testing identified Epstein-Barr virus and HHV-7, leading to antiviral treatment followed by empiric multidrug therapy despite negative results for other pathogens. Although tuberculosis therapy partially improved symptoms, mNGS ultimately identified neurobrucellosis, enabling appropriate treatment and clinical recovery.

Diagnosing pediatric meningitis and encephalitis is challenging due to overlapping symptoms such as fever and pleocytosis, frequent neurologic complications, and limited CSF volumes in neonates and children. As a result, clinicians often rely on repeated testing and prolonged empiric therapy. mNGS can rapidly identify the cause of unexplained neurological symptoms in children using a single lumbar puncture, enabling timely, targeted treatment.



11-YEAR-OLD GIRL
PAIN, NAUSEA
AND VOMITING,
ABNORMAL
MUSCLE
MOVEMENTS

- Previously healthy
- Presented with headache, vomiting, abnormal movements

ALL MICROBIOLOGY
TESTING NEGATIVE

Bacterial cultures
Fungal cultures
Mycobacterial culture
CMV DNA quantitative PCR
EBV DNA quantitative PCR
Enterovirus RNA
HSV-1 and HSV-2 PCR
HHV-6 PCR
HIV RNA quantitative PCR
HBV DNA quantitative PCR
DNA
Meningoencephalitis antibody
VDRL
Cryptococcal antigen
Coccidioides IgG/IgM
HIV Ab
Mycobacterium tuberculosis-quantiferon gold
Q fever antibody
RPR
Brucella, IgM antibody
• Brucella spp was not isolated from either CSF or blood culture.
• Brucella DNA was not detected in clinical 16S rRNA gene sequencing

2015

Admission (initial hospital)

Abnormal jerking movements of her fingers, hands, arms, and legs
Brucella IgM antibody negative; lumbar puncture EBV, HHV-7 positive
Acyclovir followed by oral ganciclovir

FEBRUARY 2015

Admission (second hospital)

Second lumbar puncture, tested negative for various pathogens
Empiric 4-drug therapy for Mycobacterium tuberculosis initiated with isoniazid, rifampin, pyrazinamide, and ethambutol based on risk factors

DAY 8

Third lumbar puncture

Tests for Mycobacterium tuberculosis complex were negative
Treatment modified for possible multidrug-resistant TB by adding levofloxacin and replacing ethambutol with ethionamide

DAY 39

Discharged without dx

Persistent symptoms of fatigue, mild back pain, and intermittent episodes of shaking of extremities
Continued 5-drug antimycobacterial regimen

MARCH 2016

mNGS detects neurobrucellosis in CSF

Patient re-admitted after contacted with mNGS results and instructed to seek further medical evaluation
Doxycycline and rifampin
Symptoms fully resolved

CASE 5

mNGS could yield faster diagnosis for immunocompromised patients at greater risk for complications and death

A 68-year-old man on chemotherapy for treatment of mantle cell lymphoma developed fever, chills, fatigue, and dizziness. Lung imaging revealed inflammatory pneumonitis. Empirical therapy was started, but the patient deteriorated. All microbiologic test results for CSF, blood, and bronchoalveolar lavage were negative. An mNGS test was performed on the

patient as part of a research study, and the results revealed the presence of St. Louis encephalitis Virus (SLEV). Routine diagnosis of SLEV is challenging because serologic testing is only performed by specialized reference laboratories, the period of viremia is brief, and molecular testing by reverse transcription PCR is not widely used.



68-YEAR-OLD MAN

- Immunocompromised
- Coronary artery disease
- Hypertension
- Mantle cell lymphoma
- Presented with fever, chills, fatigue, dizziness

ALL MICROBIOLOGY TESTING NEGATIVE

Blood
Bacterial cultures
Fungal cultures
Mycobacterial culture
Aspergillus antigen EIA
Adenovirus PCR
CMV DNA quantitative PCR
EBV DNA quantitative PCR
Enterovirus RNA
HSV-1 and HSV-2 PCR
HHV-6 PCR
HIV RNA quantitative PCR
HBV DNA quantitative PCR
Leptospira DNA
Parvovirus B19 DNA
VZV DNA, qualitative PCR
Cryptococcal antigen

CSF
HSV 1&2 PCR
Fungal culture
Bacterial culture
Coccidioides Ab CF, ID
CMV PCR
EBV PCR
HHV-6 PCR
JC polyomavirus DNA, PCR
Mycobacterial culture
MTb DNA
Meningoencephalitis antibody
VDRL
VZV Ab IgG
West Nile virus RNA
mNGS for pathogen detection

Respiratory Secretion Testing
Bacterial culture
Fungal culture
Respiratory virus panel
Mycoplasma pneumoniae PCR
HSV-1 and HSV-2 PCR
CMV PCR
Pneumocystis DFA
Mycobacterial culture
Legionella culture and urinary Ag

Serological Testing
Coccidioides IgG/IgM
Coccidioides complement fixation
HCV Ab
HBV (core Ab and hepatitis B e Ab)
Mycobacterium tuberculosis-quantiferon gold
Q fever antibody
RPR
SLEV, IgM/IgG antibody

2016

Admitted

Fever, chills, fatigue, dizziness
Brain MRI and EEG negative; CT/PET imaging shows inflammatory pneumonitis
Vancomycin, meropenem, levofloxacin

DAY 3

Transferred to ICU

Anemic, acutely hypoxic
Intubation, lumbar puncture reveals CSF pleocytosis.
Acyclovir and antifungal therapy were added to initial regimen

DAY 9

Repeat lumbar puncture

Lumbar puncture shows persistent pleocytosis, microbiologic test results for CSF, blood, and bronchoalveolar lavage negative
mNGS detects St. Louis encephalitis virus (SLEV)

DAY 23

Transferred to comfort care

Poor prognosis, bronchopneumonia

DAY 24

Patient dies

Patient's prognosis declined rapidly, as rounds of microbiological testing were performed.

CASE 6

mNGS solves long-standing medical mystery

Culture and PCR remain first-line diagnostic tools, but these conventional diagnostics are limited. They can only help confirm the presence of already-suspected pathogens. When those or serology tests come up negative, suspicion of CNS infection can remain high in patients with fever, altered mental state, flu-like symptoms, rash, and elevated white blood cell counts or other inflammatory markers. These patients are ideal candidates for mNGS testing.

A 26-year-old woman presented with low back pain followed by saddle anesthesia (groin numbness) and unilateral foot drop. Extensive infectious disease diagnostic studies were initially unrevealing. Three months later, another lumbar puncture was performed to obtain CSF. mNGS analysis of the second sample revealed the presence of *Candida dubliniensis* allowing targeted treatment and clinical recovery.



26-YEAR-OLD
WOMAN
PERSISTENT
LOWER BACK PAIN

- history of migraines
- initially undisclosed history of injection drug use
- Presented with altered mental status, flu-like symptoms, rash and high white count, lower back pain

ALL MICROBIOLOGY TESTING NEGATIVE

Extensive infectious disease diagnostic studies
Pathology findings no microbes.
18s rRNA PCR test
16s rRNA PCR test

2015

Presentation with lower back pain

JAN 2017

Groin numbness, foot drop

Cisternal CSF: 126 WBC/mm³ (67% neutrophils, 22% lymphocytes, 11% monocytes), 4 RBC/mm³, total protein: 105 mg/dL, glucose: 40 mg/dL

MARCH 2017

MRI, lumbar meningeal biopsy

Negative: extensive infectious disease diagnostic studies, CSF and tissue biopsy
18s rRNA and 16s rRNA PCRs

JULY 2017

Wheelchair bound

AUGUST 2017

mNGS identifies *Candida dubliniensis*

Treated with amphotericin and flucytosine
Regained motor function, decreasing leptomeningeal enhancement on MRI

CASE 7

Culture-negative coccidioidal meningitis in a teenager with behavior changes

An adolescent, previously healthy with a recent history of fever, cough and congestion, began showing signs of cognitive and balance issues. The teenager exhibited signs of memory impairment, double vision, and phrase repetition, and reported headaches. Because the diagnostic CT and EEG were negative, the patient was sent home. Symptoms worsened and patient was re-admitted. MRI hinted at TB, fungal infections and sarcoidosis.

Lumbar puncture yielded low volume, revealed elevated opening pressure, high protein and white blood counts, and low glucose levels. Patient was started on empiric treatment with vancomycin, ceftriaxone, and fluconazole. mNGS identified *Coccidioides* meningitis in less time than standard diagnostic tests, allowing clinicians to immediately change treatment to antifungal therapy and fluconazole maintenance was initiated.



TEENAGER
MEMORY AND
BALANCE ISSUES

- Previously healthy
- Recent history of fever, cough, congestion

2015

Admitted, Discharged

Headache, repeating phrases, memory deficits, double vision
CT, EEG performed at outside hospital negative
Sent home

JAN 2017

Re-admitted

Worsening symptoms
MRI with bibasilar enhancement

MARCH 2017

Lumbar puncture

Low-volume lumbar puncture: high opening pressure, elevated protein and WBC, low glucose levels
Treated with empiric vancomycin, ceftriaxone, and fluconazole

DAY 4

mNGS identifies *Coccidioides* spp.

Treatment changed to antifungal Amphotericin B

DAY 6

Serum *Coccidioides* IgG positive

Fungal culture negative

DAY 15

CSF *Coccidioides* IgG positive

Fluconazole maintenance

CASE 8

Diagnosis of novel human arbovirus (Potosi) in immunocompromised patient with neurologic decline

A woman in her 30s with relapsing–remitting multiple sclerosis, treated with immunosuppressants, developed a slow but progressive neurologic decline over several months. Her initial symptoms began after a cruise to the Bahamas and included confusion and subtle word-finding difficulty, followed by worsening cognitive processing and gait impairment. She had no systemic symptoms at that time. An initial hospitalization included lumbar puncture and diagnostic testing, which were negative.

Over time, her condition deteriorated. She was later re-admitted with fevers and profound neurologic decline. She was nonverbal and catatonic, raising concern for malignant catatonia. Despite treatment with electroconvulsive therapy, including six sessions resulting in an induced coma, her neurologic status did not improve. She was transferred to an academic medical center for further evaluation.

Repeat CSF analysis showed only two white blood cells with normal protein and glucose levels. Several brain MRIs showed

months of progressive, global central nervous system atrophy, without a clear explanation. Given the continued decline and lack of response to therapy, patient was ultimately transitioned to hospice.

Advanced diagnostic testing using mNGS identified Potosi virus, a mosquito-borne arbovirus not previously reported in humans. This discovery, reported in 2025, prompted a CDC investigation into Potosi virus as a potential emerging infectious disease.



FEMALE IN 30s
NEUROLOGIC
DECLINE

- Relapsing/remitting multiple sclerosis
- Immunosuppressants

FOUR MONTHS

Mental confusion

Cruise to Bahamas, patient develops illness with subtle word-finding difficulties

Symptoms worsen

Patient develops gait issues, but no systemic symptoms

Patient develops gait issues, but no systemic symptoms

Lumbar puncture: negative
Discharged

Re-admitted

Fevers, non-verbal, catatonic
Suspected malignant catatonia
Treated with progressive electroconvulsive therapy; induced coma
Transferred to academic center
MRI review showed months of progressive global CNS atrophy

Lumbar puncture

CSF LP: negative

Hospice

Patient provided supported care

mNGS identifies Potosi

Mosquito-borne virus previously unreported in humans was diagnosed by mNGS, prompting CDC to investigate as an emerging infectious disease.



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