

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). [🔗](#)

Limitations of Traditional Testing Methods Techniques

- Blood cultures miss $\geq 70\%$ 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. [🔗](#)

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. [🔗](#)

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.