# CNS Co-infection with *Aspergillus terreus* and Varicella-Zoster Virus Identified by Metagenomic Next-Generation Sequencing of CSF in an Immunocompromised Host

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AMP 2025
Case Studies in Infectious Diseases
Nov 14 2025

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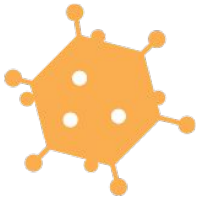
# Disclosures

SD, SM: Employee, shareholder, Delve Bio

# Meningitis and Encephalitis: Diagnostic and Stewardship Challenges

**60%**

of cases are due to infectious pathogens



**50%**

of cases remain undiagnosed

**>100**

different pathogens linked to ME

## Empiric antimicrobial use

**86%** antibiotics

**53%** antivirals

**8%** antifungals

## Cost per patient

**$64k-260k**

## ICU level care

**22%**


Ellul M, *Lancet Infect Dis*, 2022
Glaser CA, *Clin Infect Dis*, 2006
Granerod J, *Lancet Infect Dis*, 2010
Mailles A, *Clin Infect Dis*, 2009
Vora NM, *Clin Infect Dis*, 2017

# Delve Detect CSF: Test Overview and Controls

## Test Description

- Delve Detect CSF is an mNGS assay for qualitative pathogen detection in CSF from patients with suspected meningitis or encephalitis
- Detects organisms by sequence homology of nucleic acid (DNA & RNA libraries)
- Sequencing on Illumina platform
- Proprietary bioinformatic pipeline for read assignment
- Reports reviewed by board-certified physicians with interpretation and comments
- Results should be considered in the context of clinical history, labs, and imaging

## Controls

- Negative control: Synthetic CSF matrix
- Positive control: Synthetic CSF with 7 representative non-pathogenic organisms (DNA & RNA viruses, GN / GP bacteria, fungi, parasites)
- Internal control: DNA and RNA phage spiked into each sample prior to extraction

# Clinical use cases for CSF mNGS testing

| | |
|---|---|
| **Expedited Diagnosis in High-Risk Patients** | • Critically-ill, ICU<br>• Immunocompromised hosts<br>• Pediatric patients |
| **Resolve Atypical Clinical Presentations** | • Suspected atypical or fastidious pathogen<br>• High clinical suspicion with negative conventional testing |
| **Optimize Treatment Decisions** | • Antimicrobial de-escalation<br>• Initiation of immunosuppression<br>• Tissue-localized brain infections |
| **Public Health and Geographic Medicine** | • Unusual pathogens<br>• Complex exposure history<br>• Outbreak detection and PH investigations |

# Role of CSF mNGS in CNS infections

- Immunocompromised hosts with CNS lesions often have overlapping infectious and non-infectious etiologies
- Traditional CSF culture and PCR panels may fail to identify opportunistic infections or co-infections
- mNGS offers comprehensive, unbiased and simultaneous detection of both DNA and RNA pathogens, offering clinical value in rapidly deteriorating, diagnostically complex patients

# mNGS Workflow: Laboratory + Bioinformatics

## Sample Processing and Sequencing

### Optimized for sensitivity, specificity, and contamination-free results


**CSF sample**
Stored in stabilization buffer

**Lysis and Purification**
DNA + RNA from all organisms

**Target Enrichment**
RNA & DNA viruses, bacteria, fungi, parasites

**Library Prep**
DNA and RNA libraries

**Amplification and Pooling**

**Sequencing**

## Bioinformatics Pipeline (Delve D-CIDE)

### Optimized for speed and removal of non-microbial background

**Preprocessing**

**Host Background Removal**

**Microbial Identification**

**Pathogen Classification**

**Automated Reporting**

**Clinical Consultation**

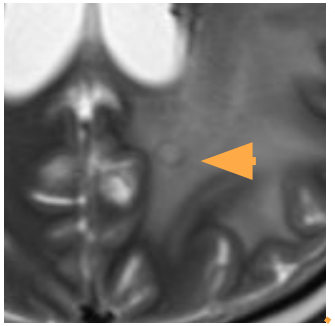
# Clinical history + initial exam

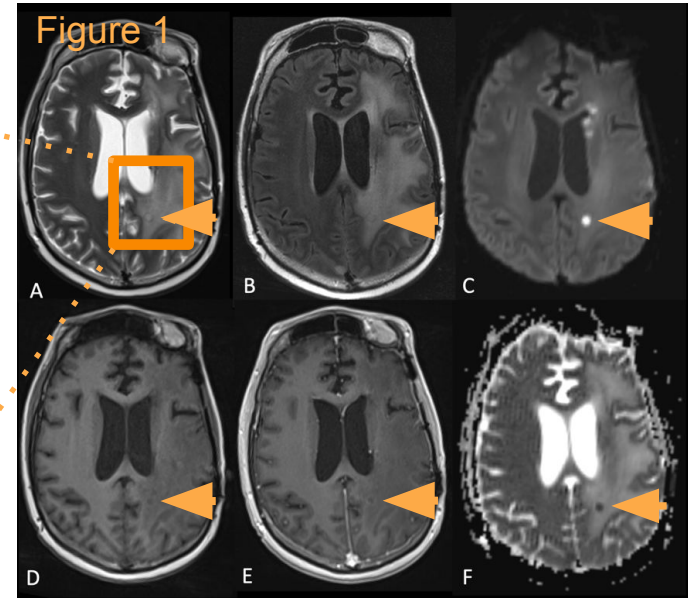
- 37-year-old woman with aggressive glioblastoma and leptomeningeal spread on high-dose corticosteroids + bevacizumab
- Presented with 2 days of fever and acute altered mental status
- Exam: Lethargic, non-verbal, diminished right-sided breath sounds.
- Blood cultures: *Streptococcus pneumoniae*, treated appropriately with antibiotics
- Chest CT: Dense right-lower-lobe pneumonia with central necrosis → concern for hematogenous spread

# Continued workup

- MRI brain: Multiple diffusion-restricting, non-enhancing lesions → rapid progression over 48h
  - DDx: Septic emboli vs invasive fungal infection
- CSF: 120–161 WBC/µL (90 % PMNs), protein 90 mg/dL; Gram stain / culture negative
- Serum: β-D-glucan > 500 pg/mL; galactomannan positive.
- Started on voriconazole + caspofungin for possible disseminated fungal infection
- CSF sent for Delve Detect CSF mNGS

# Brain MRI





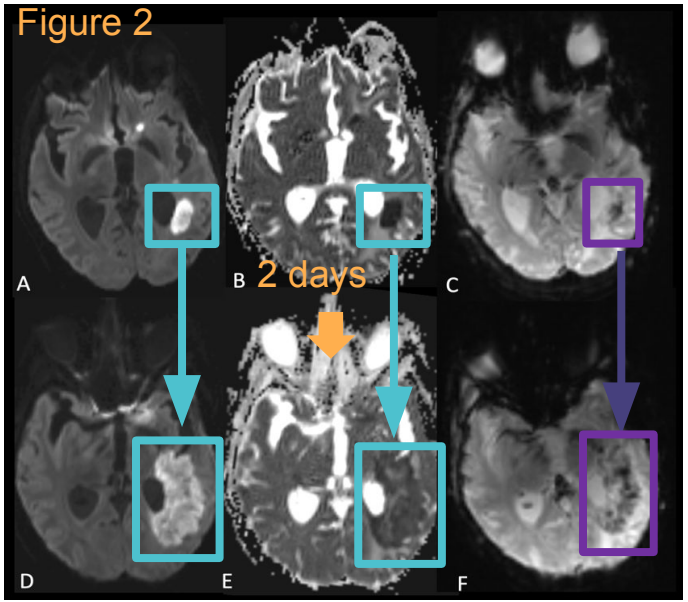
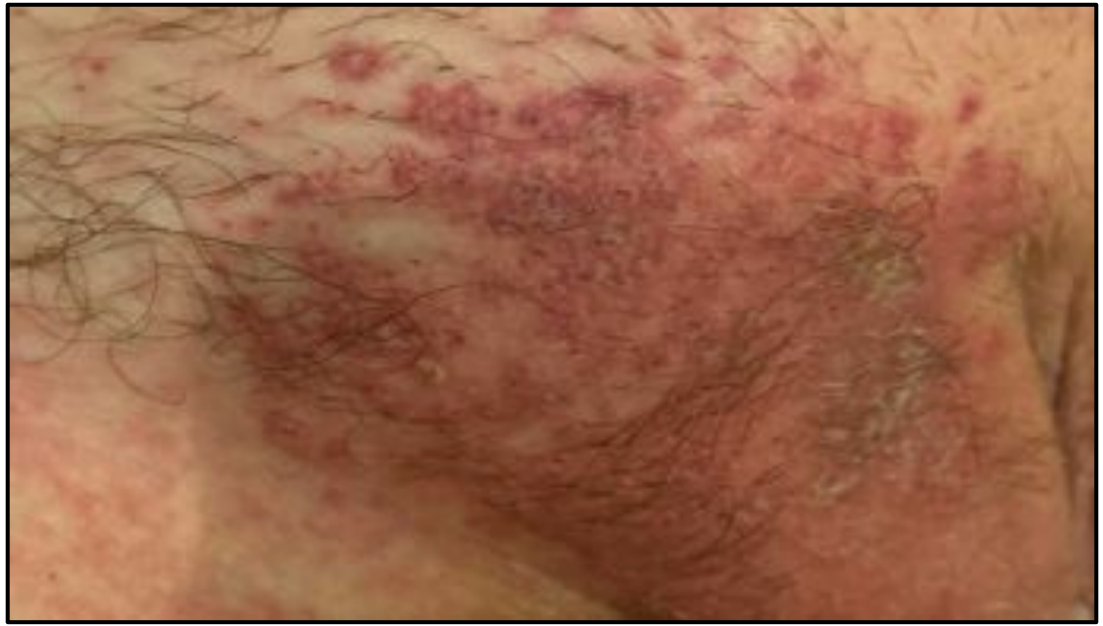




**Figure 1 –** Initial brain MRI. (A) T2, (B) T2/FLAIR, (C) DWI, (D) T1 pre-contrast, (E) T1 post-contrast, (F) ADC, axial views. In the left parietal lobe, there is "target sign" (orange arrow) characterized by central hyperintensity, a variably hypointense middle ring, and a hyperintense outer rim *(zoomed-in),* which is is an independent marker of cerebral aspergillosis.

**Figure 2.** Initial (A–C) and follow-up (D–F) brain MRIs obtained 2 days apart. (A,D) diffusion-weighted imaging (DWI), (B,E) apparent diffusion coefficient (ADC), and (C,F) susceptibility-weighted imaging (T2*), axial views. Comparison of the initial MRI (top) with the follow-up MRI (bottom) obtained 2 days later shows marked interval increase in the extent of restricted diffusion (blue box) and susceptibility artifact (purple box). Although restricted diffusion with low ADC values in glioblastoma patients receiving bevacizumab is often associated with bevacizumab-related coagulative necrosis, a rapid progression is atypical for this process and should always raise strong concern for a **superimposed invasive fungal infection**. Fungal hyphae can accumulate paramagnetic metals such as iron, manganese, and magnesium, which cause local magnetic field inhomogeneity, leading to *T2/SWI signal dropout* due to accelerated spin dephasing. Overall, the findings are most compatible with **cerebral aspergillosis**.

# Cutaneous findings



Clinical photograph of the right groin demonstrating an erythematous rash with scaling and early vesicular change, subsequently confirmed positive for VZV by PCR.

# mNGS results

## Table A - Organism Result Summary

| Organism | Total reads | Unique reads | rpM | rpM ratio | Biomass (fg) | Confidence notes |
|---|---|---|---|---|---|---|
| **Aspergillus terreus** | 4773 | 3130 | 148.26 | 148.26 | 9676 | BLAST-confirmed; genomic alignment support; high biomass signal |
| **Varicella-Zoster Virus (VZV)** | 1681 | 1159 | 52.21 | 52.21 | 3408 | BLAST-confirmed; genomic alignment support; high genomic coverage and biomass signal |

## Table B - Sequencing & Host Background Metrics

| Library | Reads PF | % Human | Total biomass (fg) | Control biomass (fg) | Human biomass (fg) |
|---|---|---|---|---|---|
| **DNA** | 32,194,356 | 99.798 | $1.308 \times 10^{9}$ | 62,440 | $1.307 \times 10^{9}$ |
| **RNA** | 44,824,162 | 72.23 | $1.118 \times 10^{7}$ | 25,770 | $1.114 \times 10^{7}$ |

# BLAST summary VZV (top matches)

| Rank | Organism (hit) | Accession | Max score | Query cover | E-value | % identity |
|---|---|---|---|---|---|---|
| 1 | Human alphaherpesvirus 3 isolate KPZ12-283, complete genome | MH709324.1 | 204 | 100% | 2.0E−48 | 100.00% |
| 2 | Human alphaherpesvirus 3 isolate pps-p60, complete genome | MW545808.1 | 204 | 100% | 2.0E−48 | 100.00% |
| 3 | Human herpesvirus 3 isolate T25, partial genome | KF558379.1 | 204 | 100% | 2.0E−48 | 100.00% |
| 4 | Varicellovirus humanalpha3 isolate UGA-VZV-042, complete genome | PV845293.1 | 204 | 100% | 2.0E−48 | 100.00% |
| 5 | Human herpesvirus 3 isolate v76, partial genome | KF558380.1 | 204 | 100% | 2.0E−48 | 100.00% |

**Interpretation:** Multiple top hits align to *Varicellovirus humanalpha3* (Human alphaherpesvirus 3, VZV) with 100% identity and E-values of 2e−48, confirming species-level identification with complete or near-complete genome matches.

# BLAST summary *Aspergillus terreus* (top matches)

| Rank | Organism (hit) | Accession | Max score | Query cover | E-value | % identity |
|---|---|---|---|---|---|---|
| 1 | Aspergillus terreus NIH2624 uncharacterized protein (ATEG_04416), partial mRNA | XM_001213594.1 | 204 | 100% | 2e−48 | 100.00% |
| 2 | Aspergillus citrinoterreus strain CBS138921 chromosome VI | CP155514.1 | 193 | 97% | 5e−45 | 99.07% |
| 3 | Aspergillus fischeri NRRL 181 trichodiene synthase (TRI5) domain protein (NFIA_042070), partial mRNA | XM_001266525.1 | 182 | 100% | 1e−41 | 96.36% |
| 4 | Aspergillus campestris IBT 28561 trichodiene synthase (P168DRAFT_261906), partial mRNA | XM_024834716.1 | 139 | 98% | 2e−39 | 89.81% |
| 5 | Diplodia seriata uncharacterized protein (SLS55_000001), partial mRNA | XM_066771518.1 | 97.1 | 85% | 4e−16 | 85.26% |

**Interpretation:** Top BLAST hits align to *Aspergillus terreus* with 100% identity and E-values of 2e−48, confirming species-level identification. Lower-identity matches to other *Aspergillus* species and distant hits to *Diplodia* indicate specificity for *A. terreus* as the dominant fungal signal.

# Clinical impact

- Rapidly progressive CNS disease in an immunocompromised host with negative CSF cultures
- mNGS detected dual infection with *Aspergillus terreus* and VZV
  - Confirmed invasive mold CNS infection, revealed unrecognized viral co-infection
- Guided timely addition of acyclovir and continuation of antifungal therapy within ~30 hours
- Findings reported within ~30 hours, directly influencing management and avoiding further invasive diagnostic procedures

# Key takeways

- Early mNGS can detect opportunistic and clinically unrecognized co-infections missed by conventional testing in immunocompromised hosts
- Species-level identification for fungal pathogens enables precise antifungal selection
- Early implementation of mNGS in diagnostically urgent CNS presentations can change treatment course and improve outcomes

# Thank you!

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