

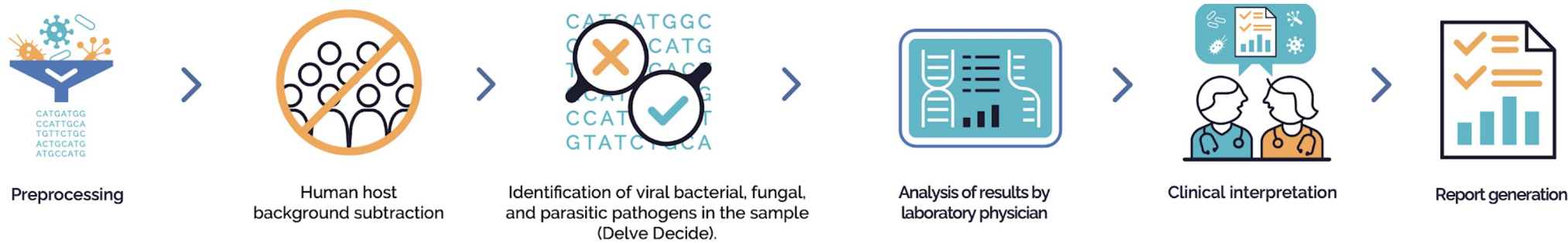
Enterovirus Subtyping using Unbiased Metagenomic Next-Generation Sequencing for Patients with Enteroviral Meningitis



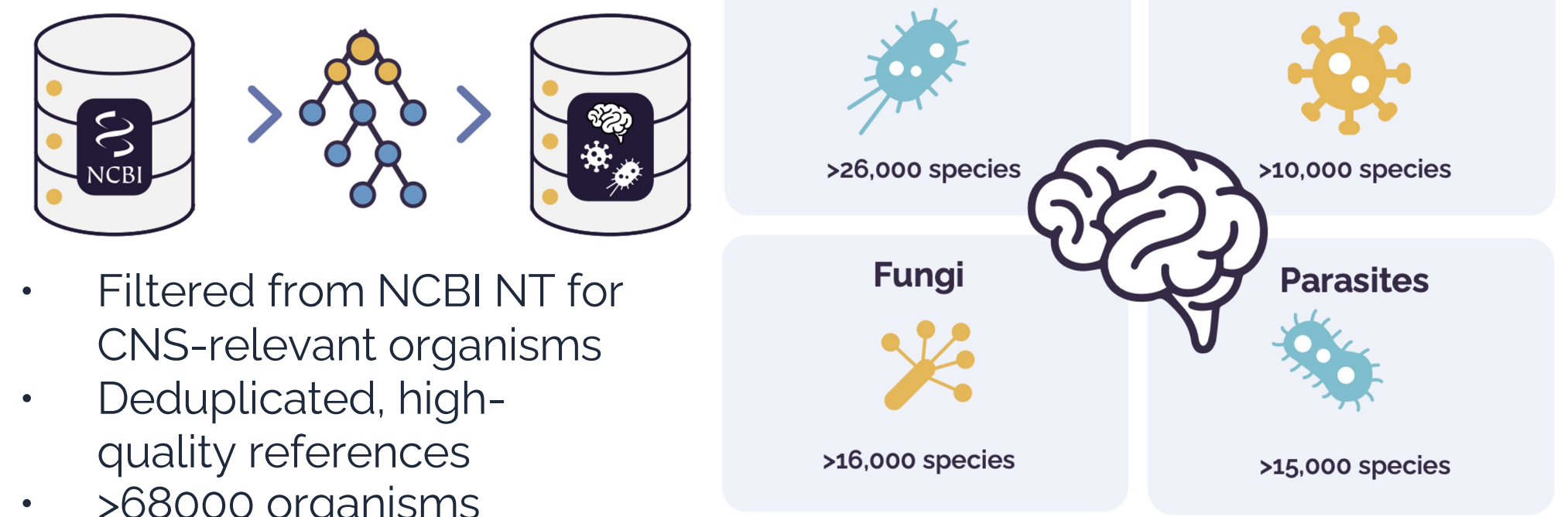
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Pathogen Detection Through mNGS



Curated Pathogen Database



Background

- Enterovirus (EV) is a RNA picornavirus that can cause a variety of central nervous system manifestations in susceptible patients
- Metagenomic next-generation sequencing (mNGS) provides sensitive detection and simultaneous strain typing
- EV subtype analysis can provide information about potential clinical severity and epidemiology to influence treatment options

Objectives

- Establish the performance characteristics of EV subtyping in clinical CSF samples
- Correlate EV subtype information with clinical manifestations and therapeutic management

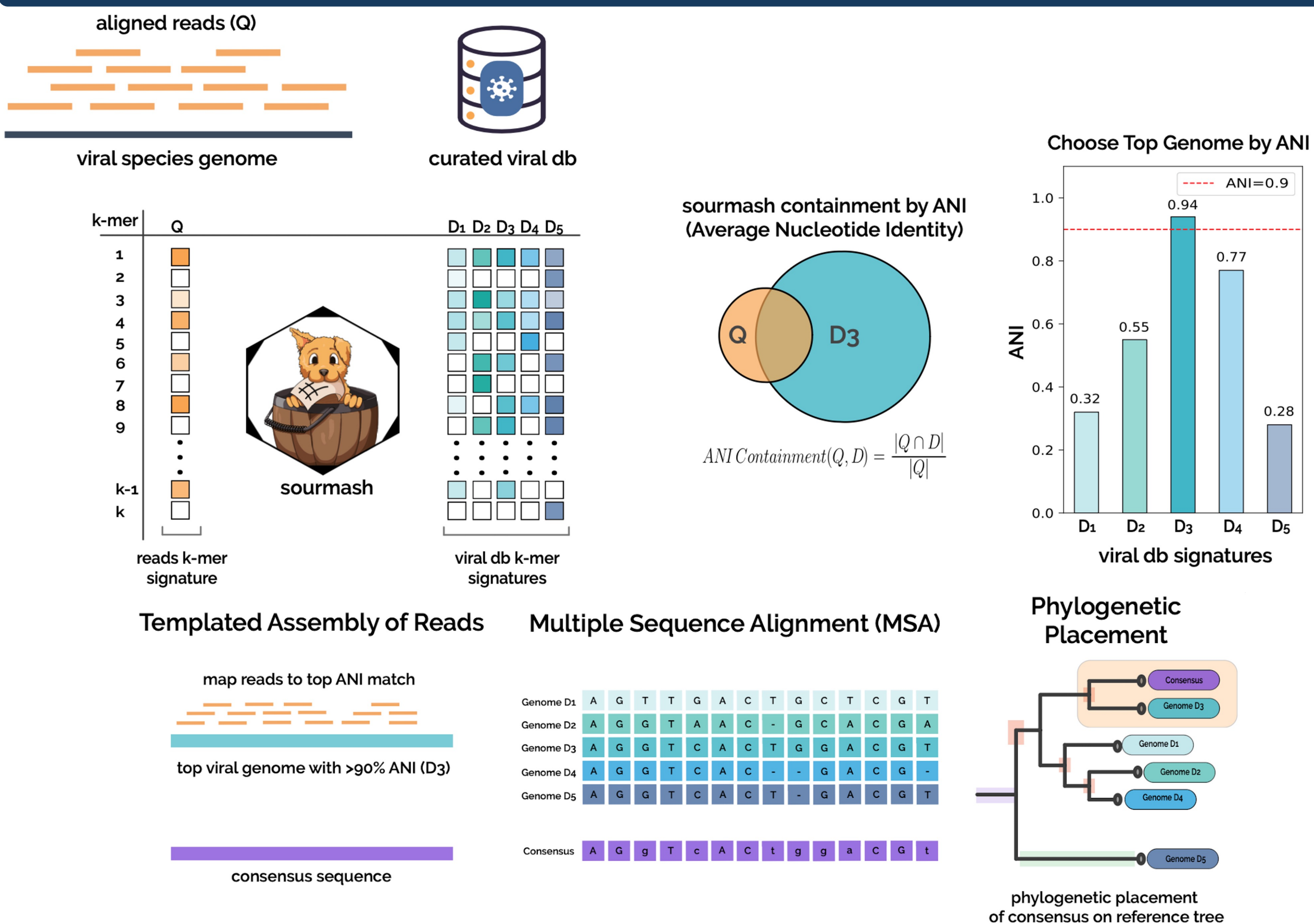
Methods

- CSF mNGS with EV subtyping performed, results compared to reference standard EV PCR-sequencing
 - 41 *in silico* sample datasets
 - 7 Contrived samples
 - 16 patient CSF samples
- Multicenter case identification with retrospective chart review

CNS Enterovirus Infection Cases

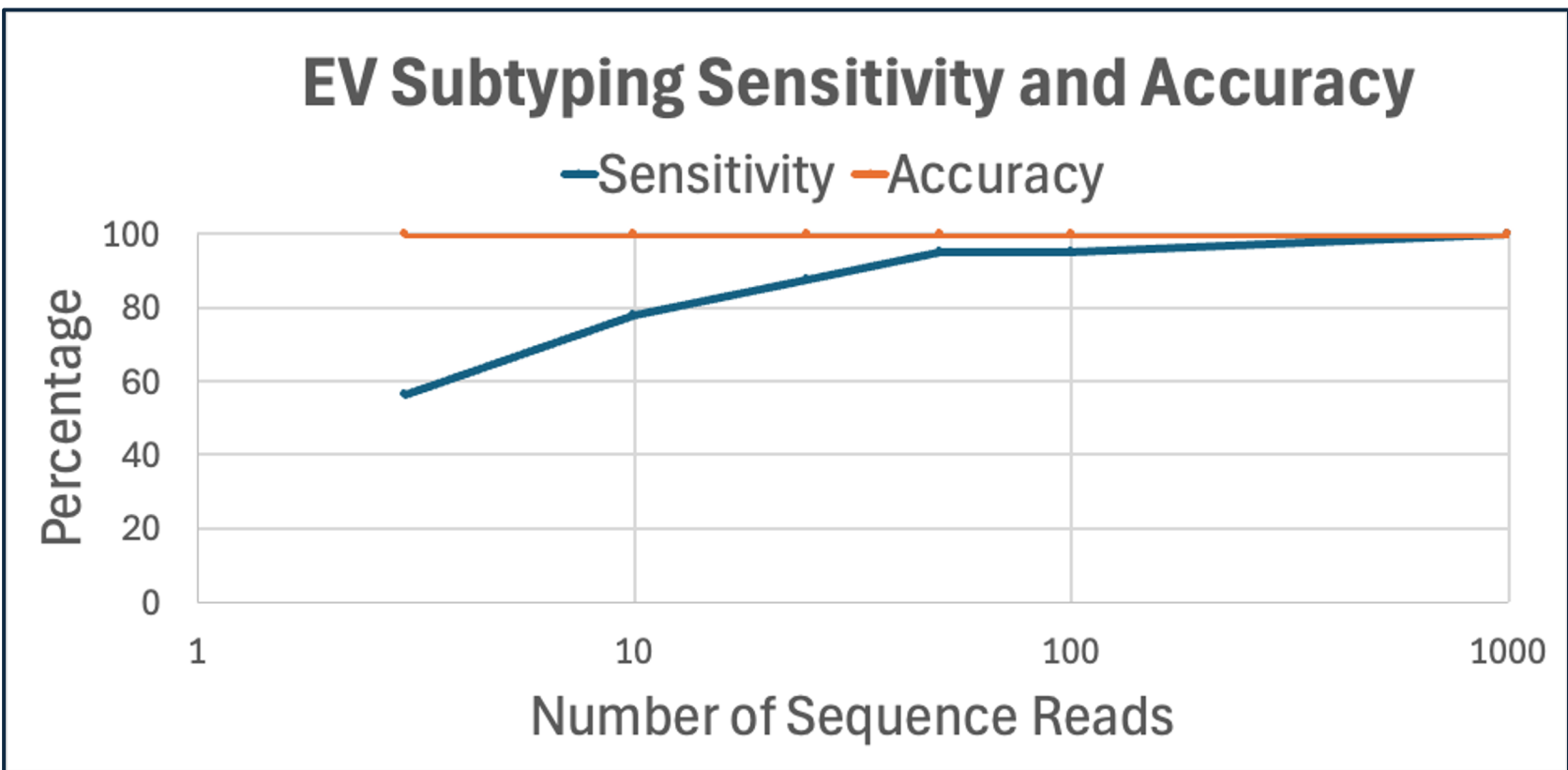
Patient Type	Clinical Scenario	EV Subtype	Orthogonal CSF EV PCR	Clinical Impact
Adult	Follicular lymphoma on Rituximab, multiple admissions with AMS	Enterovirus A71	Positive	IVIG + Pocapavir
Adult	Multiple sclerosis on Ublituximab with encephalopathy	Coxsackievirus A16	Positive	IVIG
Adult	Multiple sclerosis on Rituximab with flaccid paralysis	Coxsackievirus A16	Positive	Supportive management
Adult	Systemic lupus erythematosus w/ myocarditis on Rituximab, prior respiratory EV+ 4 months prior	Coxsackievirus B3	Positive	Supportive management
Adult	Multiple sclerosis on Ocrelizumab with cerebellitis	Coxsackievirus B1	Positive	IVIG + Pocapavir
Infant	Neonatal sepsis / meningitis	Echovirus E11	Positive	Supportive management
Infant	Neonatal sepsis / meningitis	Echovirus E11	Positive	Supportive management
Adult	Multiple sclerosis on Rituximab, Hospitalized 6 months prior with EV encephalitis, recurrent admission	Indeterminate	Negative	High Dose IVIG

Enterovirus Subtyping Bioinformatic Method



mNGS EV Subtyping Performance

Short Read Archive (SRA) *in silico* Analysis



Modeling of 41 SRA EV sample datasets with known subtypes demonstrates 100% accuracy when confidence metric (p-value < 0.05) is achieved. The ability to accurately subtype improves as the number of mNGS sequence reads increases with 95% sensitivity at 50 reads. Subtypes included: EV-A: 9, EV-B: 21, EV-C: 7, EV-D: 4.

Clinical CSF Sample Analysis

Sample Type	Samples	Sensitivity (%)	Accuracy (%)
Clinical CSF	16	14/16 (87.5%)	100%
Contrived CSF	7	7 (100%)	100%
Total	23	21 (91.3%)	100%

EV Subtyping of clinical CSF samples and spiked CSF samples with known subtypes. All samples with confidently called subtypes were concordant with the reference result. Subtypes included: EV-A: 6, EV-B: 6, EV-C: 0, EV-D: 1.

Conclusions

- Detection of EV by mNGS is highly concordant with EV PCR but appears to have higher sensitivity in some chronic infection cases
- EV subtyping based on untargeted mNGS data provides accurate results with sufficient read coverage
- Several cases of severe / prolonged EV infection were seen in patients on B-cell depleting therapy
- EV subtyping can inform prognosis and treatment options for chronic infections, including IVIG and investigational pocapavir therapy