Development of a Rapid and Cost-Effective NGS-based Assay for the Clinical Diagnosis of Meningitis and Encephalitis

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Introduction

Uncovering the etiology behind complex cases of meningitis and encephalitis is time-consuming and complex, with nearly 50% of cases going undiagnosed. The current standard of care offers limited diagnostic options and often utilizes costly serial testing, particularly when an infectious pathogen is suspected. Metagenomic next generation sequencing (mNGS) offers the ability to sequence and detect many pathogens agnostically, but hurdles like scaling, costs, and turnaround times persist.

Delve Bio and Broad Clinical Labs (BCL) collaborated on a rapid and scalable metagenomic NGS workflow, using cerebral spinal fluid to address current known challenges and to enable:

- 72-hour TAT from sample receipt to reporting of results
- Simultaneous processing of Illumina-compatible DNA and RNA libraries
- unbiased detection of all bacterial, viral, fungal, and parasitic pathogens extant in NCBI Nucleotide sequence repository

This process, as presented, offers rapid pathogen-agnostic screening for neurological illnesses which potentially could improve patient outcomes by reducing hospital stays and hastening time between admission and treatment.

mNGS Clinical Requirements

To ensure the clinical effectiveness in diagnosing Meningitis and Encephalitis, established targets are vital. They were used to gauge development success and to ensure marketed promises like faster treatment identification and overall cost reduction

TAT: Current methods of diagnosis involve time-consuming differential diagnostic approach, utilizing a combination of methods. Results are often delivered between 10-15 days after hospitalization.

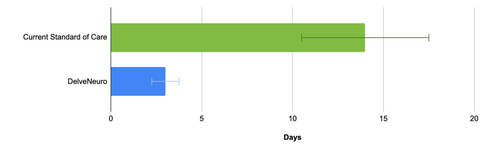

Figure 1. *Reduction in TAT between currently available clinical CSF mNGS testing and DelveNeuro*

Flexibility: As a continuously operating assay the test must be able accommodate a varying number of samples daily. This flexibility needed to be established without compromising major fluctuations in daily running costs.

Single Comprehensive Test: The assay must be designed in a way to screen for potential pathogens within a singular and simplified workflow.

Wet lab Workflow - Challenges and Solutions

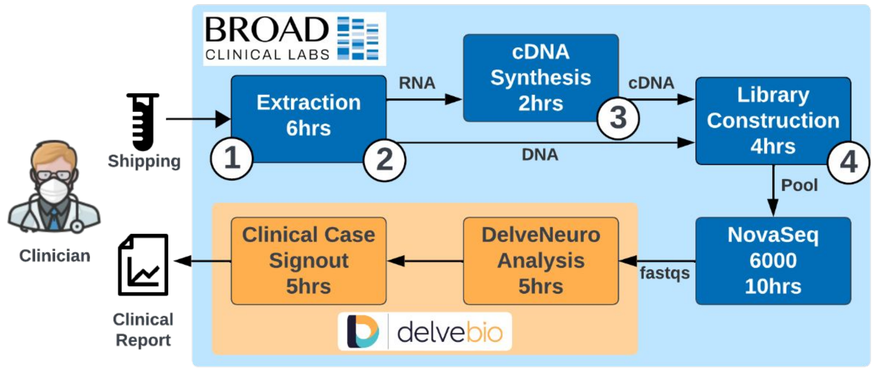

Figure 2. *Process workflow diagram annotated with step duration*

Extraction	6 Hours
cDNA Synth.	2 Hours
Lib Con.	4 Hours
NovaSeq 6000 SE 130	10 Hours
Analysis	5 Hours
Clinical Data Review	5 Hours
Total Time	**~32 Hours**

Table 1. *Cumulative processing time for end-to-end process*

Challenges for Sample Workflow

1. **Shipping and Clinical Receipt**
   - Addition of a stabilizing agent during sample collection allows for shipping specimen at ambient temperatures, which eliminates 30 mins to 1hr spent thawing samples upon receipt.
2. **Adapting to Variable Volume** - Elimination of Batch Processing and Establishing Continuous Flow
   - Extraction and library construction automation are programed for variable throughput, allowing samples to be run immediately, rather than waiting for additional samples to create a processing batch.
3. **Process Design and Simplification**
   - Consolidation of the cDNA and DNA libraries into one plate during Library Prep for ease of processing
   - Assess the time and value of each process step and eliminate unnecessary aspects
4. **Automation**
   - The use of automated liquid handlers throughout the process ensures a consistent lab processing time, reduces process variability between and within batches, and reduces the dead-volume requirements for many library construction reagents.
   - Automated liquid handlers used:
     - Hamilton Microlab STARlet
     - Agilent Bravo (LT and ST)
     - Formulatrix Mantis

Figure 3. *Various Automated Equipment used*

Unifying Analytics Workflow

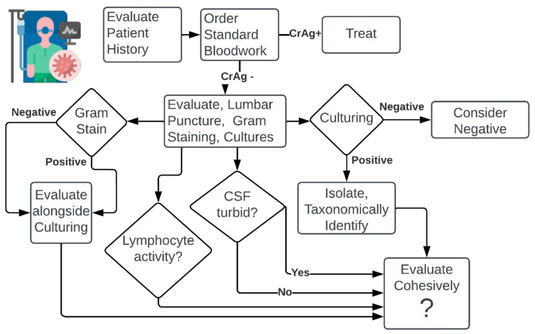

Figure 4. *Example of a differential diagnostic pathway for identifying and diagnosing a meningitis or encephalitis case. Each step in the process can take several days, and is prone to error which can lead to the wrong diagnosis and treatment.*

Figure 5. *DelveNeuro's single, hypothesis-free workflow*

Traditional diagnostic methods of central nervous system infections utilise diffuse and scattered workflows. (Figure 4.)

In contrast, Delve Neuro's diagnostic approach provides a single, broad, hypothesis-free test that aligns patient samples to a curated sequence database of bacterial, viral, fungal, and parasitic pathogens for a comprehensive taxonomic profiling and pathogen identification.

Summary

BCL and Delve Bio have designed a high throughput sample processing and analysis workflow that significantly reduces turnaround time compared to classical differential diagnosis and current mNGS assays by several days. By leveraging automation end-to-end, we have ensured that processing times are consistent, and waste is reduced, which helps keep cost low despite a variable throughput non-batched process. The workflow has been designed with the ability to run up to 150 samples per week without major changes to the workflow.

Utilizing a highly curated database of pathogens, The DelveNeuro computational pipeline robustly identifies causal and clinically relevant pathogens of interest from sequencing outputs of lumbar punctures in a single, comprehensive test.

This workflow has the potential to improve patient outcomes and reduce associated healthcare costs by reducing the time between patient admission and diagnosis, thereby ensuring appropriate therapies are initiated rapidly and effectively.

Underlying methods references

1. Miller S, et al. 2019 "Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid.", Genome Resource
2. Boers SA, et al. 2019 "Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory." European Journal of Clinical Microbiology & Infectious Diseases
3. Chiu CY, Miller SA. 2019 "Clinical metagenomics." Nature Reviews Genetics.

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