Supplementary Online Content

Diagnosis of Neurological Infections in Pediatric Patients from Cell-Free DNA Specimens using Metagenomic Next-generation Sequencing

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eMethods

eFigure 1. Contingency tables for the clinical diagnosis with cfDNA mNGS and conventional methods sets, respectively.

eTable 1. The comparison between the true positive (TP) and false negative (FP) mNGS results.

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Cell-free DNA Extraction

CSF samples were collected through centrifugation at 1600×g for 10 min, followed by centrifugation at 16,000 ×g for 10 min at 4℃. According to the manufacture's instruction for the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany), cfDNA was extracted and purified. The quality and concentration of DNA samples were monitored by a Qubit Fluorometer (Thermo Fisher Scientific, MA, USA).

Whole-cell DNA Extraction

The 124 CSF samples were homogenized using grinding instrument, TGrinder H24R (TIANGEN, Beijing, China) and centrifuged at 10,000g for 5 min. The supernatant was used for wcDNA extraction according to the manufacture's instruction for the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany). The quality and concentration of DNA samples were also monitored by a Qubit Fluorometer (Thermo Fisher Scientific, MA, USA).

Metagenomic next-generation sequencing and analysis

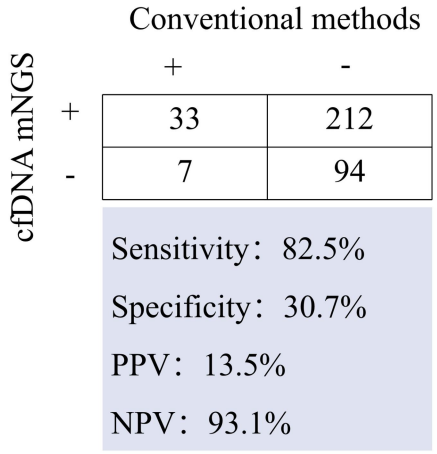
Metagenomics libraries were constructed by QIAseq Ultralow Input Library Kit (QIAGEN, Hilden, Germany) according to its manual. The qualified library was sequenced on Nextseq 550 platform (Illumina, San Diego, USA). In parallel with the clinical samples, positive control and negative control (non- template control, NTC) were also set for mNGS detection with the same procedure and bioinformatics analysis.

After filtering out adapter, low-quality, low-complexity, and shorter reads of <35bp(1), high-quality sequencing data were generated. Next, human reads were removed by mapping reads to human reference genome (GRCh38) using Bowtie2(2). The remaining clean data was aligned to the microbial genome database (ftp://ftp.ncbi.nlm.nih.gov/genomes/) using Burrow-Wheeler Aligner software(3). The reads number and reads per million (RPM) of each detected pathogen was calculated and the microbial composition was presented. The positive criteria for the mNGS result were set as follows:

1) For the detected bacteria (*Mycobacterium* excluded), fungi (*Cryptococcus* excluded), and parasites: a) genome coverage of the unique reads mapped to this microorganism ranked top10 of the same kind of microbes and the microorganism was not detected in the NTC; or b) RPMsample/RPMNTC was > 10 (RPMNTC≠0).

2) For viruses, *M. tuberculosis*, and *Cryptococcus*: a) the unique reads of this microbe was not detected in NTC but at least 1 specific read was mapped to species; or b) RPMsample/RPMNTC was > 5 (RPMNTC≠0).

After the prior analysis, if mNGS results were in accordance with the patient's clinical features, the detected pathogens would be considered as causative agents, or the detected organisms would be classified as non-pathogenic microbes(4).



eFigure 1. Contingency tables for the clinical diagnosis with cfDNA mNGS and conventional methods sets, respectively

eTable 1. The comparison between the true positive (TP) and false negative (FP) mNGS results.

		True positive (TP)	False negative (FN)	P value
Total (TP, n=177; FN, n=78)	Days of onset	12.3±14.9	17.1±19.2	0.0052
	Days of mNGS detection after admission	3.6±3.9	4.4±6.4	0.1321
	Days of antibiotic usage	5.9±7.5	9.1±10.5	0.0232
Bacteria (TP, n=102; FN, n=29)	Days of onset	10.0±10.5	17.0±14.6	0.0153
	Days of mNGS detection after admission	3.5±3.5	6.1±9.7	0.0415
	Days of antibiotic usage	6.0±7.5	13.0±13.6	0.0064
Viruses (TP, n=67; FN, n=42）	Days of onset	14.3±18.8	16.5±23.1	0.6399
	Days of mNGS detection after admission	3.6±4.0	3.1±2.5	0.9576
	Days of antibiotic usage	4.8±5.7	5.4±5.4	0.3508

eReference

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