**SUPPLEMENTARY MATERIALS FOR**

**Performance of metagenomic next-generation sequencing for the diagnosis of viral meningoencephalitis in a resource limited setting**

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**Word count:** abstract: 248 full text: 3513

**Keywords:** Metagenomics, Next-generation sequencing, Nanopore, MinION, meningoencephalitis

**Running title:** Metagenomic diagnosis of viral meningoencephalitis

**Supplementary Table 1.** Routine diagnostic workup during the study period at the study site

|  |  |  |
| --- | --- | --- |
| **Suspected clinical entity** | **First line diagnosis** | **Additional testing\*** |
| Tuberculous meningitis | Ziehl Neelsen stain, GenXpert and culture# | NA |
| Bacterial meningitis | Gram stain and culture |  |
| Meningoencephalitis | HSV PCR | VZV PCR and serological testing for flaviviruses (including JEV and DENV), and mumps virus |
| Cryptococcus | Lateral Flow Assay | NA |
| Auto-immune encephalitis | Not done | Anti-NMDAR encephalitis\*\* |

**Note to Table S1.** \*Up on requested by treating physicians, # using Mycobacteria Growth Indicator tubes, \*\*retrospective testing

**Supplementary Table 2:** Results of routine diagnosis, expanded PCR testing and mNGS analysis

**Supplementary Table 3**: mNGS reads obtained from DNA/RNA-virus workflows

|  |  |  |  |
| --- | --- | --- | --- |
|  | Total reads | Median | Range |
| DNA-virus workflow | 62,565,802 | 859,656 | 1,487,000 – 2,125,00 |
| RNA-virus workflow | 49,233,869 | 717,707 | 7,368 – 5,874,00 |

**Supplementary Table 4**: EV reads of PCR negative samples identical to reads found in sample(s) with a high abundance of EV reads with which they (did not) share(d) an index

|  |  |  |  |
| --- | --- | --- | --- |
| **CSF number** | **Number of unique EV reads found in PCR negative samples** | **Number of unique EV reads (%) (1)** | **Number of unique EV reads (%) (2)** |
| 17 | 2 | 1 (50) | 0 |
| 18 | 2 | 2 (100) | 0 |
| 19 | 4 | 0 | 0 |
| 20 | 1 | 0 | 0 |
| 21 | 2 | 2 (100) | 0 |
| 22 | 5 | 5 (100) | 0 |
| 23 | 13 | 4 (31) | 0 |
| 24 | 4 | 2 (50) | 0 |
| 25 | 1 | 0 | 0 |
| 26 | 20 | 5 (25%) | 0 |
| 27 | 7 | 4 (57) | 0 |
| 28 | 24 | 12 (50) | 0 |
| 29 | 22 | 0 | 0 |
| 30 | 20 | 2 (10) | 0 |
| 31 | 12 | 7 (58) | 0 |
| 32 | 4 | 2 (50) | 0 |

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**Note to Supplementary Table 4:**

(1) identical to reads found in sample(s) with a high abundance of EV reads with which it shared an index.

(2) identical to reads found in sample(s) with a high abundance of EV reads with which it did shared an index.

**Supplementary Table 5:** Result summary of mumps virus diagnostics

|  |  |  |  |
| --- | --- | --- | --- |
| **Patient ID** | **Serology** | **RT-PCR** | **mNGS** |
| 1 | Positive | Negative | Negative |
| 2 | Positive | Positive | Positive |
| 3 | Positive | Positive | Negative |
| 4 | Positive | Positive | Negative |
| 5 | Not done | Positive | Negative |