

Viral Metagenomic Analysis of Cerebrospinal Fluid from Patients with Acute Central Nervous System Infections of Unknown Origin, Vietnam

Appendix

Appendix Table 1. List of diagnostic assays conducted by the original study

Pathogens*	Assay	References
Dengue virus	ELISA	(1)
Japanese encephalitis virus	ELISA	(1)
Herpes simplex virus 1 and 2	PCR	(2)
Varicella-zoster virus	PCR	(3)
Enterovirus	PCR	(4)
<i>Neisseria meningitidis</i>	PCR	(5)
<i>Streptococcus pneumoniae</i>	PCR	(5)
<i>Hemophilus influenzae</i> type b	PCR	(5)
<i>Streptococcus suis</i>	PCR	(6)

*Diagnostic tests performed in every case as part of standard of care at participating hospitals including complete blood count, blood culture, gram/ZN smears, CSF culture and CSF examination if patients have neurologic symptoms and CNS infection is suspected.

Appendix Table 2. List of primers and probes used for PCR confirmatory

Viruses	Oligo sequence (5'→3')			Sources
	Forward	Reverse	Probe	
HBV	GGACCCCTGCTCGTGTACA	GAGAGAAGTCCACCMCGAGTCTAGA	FAM-TGTTGACAARAATCCTCACAAATACCRCAAG-TAMRA	Newly designed
Rotavirus	ACC ATC TWC ACR TRA CCC TC	GGT CAC ATA ACG CCC CTA TA	FAM-ATGAGCACAATAGTTAAAAGCTAACACTGT CAA-BHQ1	(7)
Enterovirus	CCCTGAATGCGGCTAAT	ATTGTCACCATAAGCAGCC	CY5-ACCCAAAGTAGTCGGTTCCG -BHQ3	(8)
HIV1	GGTGGAGAGCGTC	ATGCTRTCATCATYTTCTTC		(9)
Gemycircularvirus	ATGGGTRAARGTARTAGAAGAAAAGGG GTGGTAATGGTCGTCGGTATTC AGTCCTGAATGTTTCCACTCG	CTGCCTGRTGYCCYCCCACTA CCTCATCATTCGTAGTAAGCAATCTCA CAAGCGTTCCCTCGAAAATGAC		(10) Newly designed
Cyclovirus VN	GAGCGCACATTGAAAGAGCTAAA	TCTCCTCCTTCAATGACAGAAACAAC	FAM-CGADAATAAGGMATACTGCTCTAAAGSTGGCG-BHQ1	(11)
Molluscum contagiosum virus	AACCTACGCTACCTGAAGMTGGA	CAGGCTCTTGATGGTCGARATGGA		(12)

Appendix Table 3. Baseline characteristics, and CSF white cell count of patients with viruses detected by mNGS

Characteristic	Hepatitis B virus (n = 3)	Human immunodeficiency virus (n = 2)	Molluscum contagiosum virus (n = 1)	Gemycircularvirus (n = 1)
Male, no. (%)	3 (100)	2 (100)	1 (100)	1 (100)
Age (median; min-max)	42 (16–63)	57.5 (49–66)	7	12
Location				
Hue, no. (%)	0	0	0	0
Dak lak, no. (%)	0	0	1 (100)	0
Khanh Hoa, no. (%)	2 (75)	0	0	0
Dong Thap, no. (%)	1 (25)	2 (100)	0	1 (100)
3-d fever (at enrollment or last 3 days)				
Fever, no. (%); median (min-max)	3 (100); 38 (38–39)	1 (50); 39	1 (100); 38.5	0
Fever with unknown temp, no. (%)	0	0	0	1 (100)
No fever, no. (%)	0	1 (50)	0	0
Unknown, no. (%)	0	0	0	0
Outcome				
Death or discharged to die, no. (%)	0	0	0	0
Discharge with complete recovery, no. (%)	2 (75)	0	1 (100)	1 (100)
Discharged with incomplete recovery, no. (%)	1 (25)	1 (50)	0	0
Transferred to another hospital, no. (%)	0	1 (50)	0	0
Other (patient request), no. (%)	0	0	0	0
Unknown, no. (%)	0	0	0	0
CSF White Cells (cell/mm ³)	1530 (7–2590)	835 (340–1330)	70	60
Median (min-max)				

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