Diagnostic Methods

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Identification of putative meningoencephalitis-causing agents using metagenome sequencing

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We developed a standardized method for metagenome sequencing of CSF samples to detect meningoencephalitis-causing agents. First we examined different nuclease pre-treatments in order to achieve the highest reduction of human background sequences. Next, we compared different nucleic acid extraction methods and methods to reduce the amount of ribosomal RNAs. The remaining nucleic acids were amplified and sequenced via 454 pyrosequencing. To assess the sensitivity and specificity of the method we used spiked positive controls that included different RNA and DNA viruses as well as bacteria in certain concentrations. Depending on the type of genome, the sensitivity was at 10^2 to 10^5 molecules/µl. The metagenome analysis programs MG-RAST and RIEMS specifically detected the spiked organisms. Next, RNA and DNA libraries of 12 CSF samples of patients with meningoencephalitis of unknown etiology were sequenced. Many samples contained sequences of bacteriophages, Phycodnaviridae, Poxviridae, Polydnaviridae, Partitiviridae, Herpesviridae and Alloherpesviridae, which could be derived from contaminated reagents. In addition, some samples contained sequences with low E-values and high homology to insect viruses (Tetraviridae, Iridoviridae) and plant viruses (Endornaviridae) as well as sequences of marine viruses. A 468bp sequence with 84% homology to 'human circular dsDNA virus associated with alcoholic cirrhosis' and E-value of 3e-133 could be identified in one sample. Another sample contained two short reads with 53% homology to Semliki Forest virus (Togaviridae) and a different sample a short read with 93% homology to Parainfluenzavirus 5 (Paramyxoviridae). Two >400 bp reads with E-values of 0.0 and 98% homology to Human Echovirus E18 (Picornaviridae) could be identified in another sample. Finally, one sample contained 2461 reads that could be assembled into 8 contigs bigger than 500bp that showed 97-99% homology to the fungus Rhodotorula mucilaginosa with E-values of 0.0.