Abstract

The use of the MinION sequencer (Oxford Nanopore) was tested on samples prepared to simulate infectious samples. The tested procedure is to simulate work with a sample with an unknown pathogen. Therefore, a metagenomic approach was chosen.

Three kits were tested: Rapid Barcoding Sequencing, PCR Barcoding and Premium whole genome amplification. Each kit differed in duration, difficulty to prepare and in amplification of nucleic acids. In total it was chosen eight viruses with different genome lengths and with varying types of the genome (5,6 – 152 kb, ss/ds RNA, dsDNA). Ten samples were prepared to simulate different types of infection (respiratory, gastrointestinal tract and urine), and one sample contained pure water as a negative control. Before preparation of the library with Oxford Nanopore´s kits, DNase/RNase treatment was used. The viral RNA was transcribed into DNA and in chosen samples were amplificated to reach a higher concentration of nucleic acids.

Rapid barcoding sequencing kit detected all selected viruses with the highest number of viral reads (4403) with a length between 100 and 250 nt and quality coverage of viral genomes. PCR Barcoding kit detected five out of eight viruses, and the number of identified reads with a length of 100-200 nt distinctly decreased. Premium whole genome amplification kit detected only three out of eight viruses, but with the longest reads (up to 7500 nt). However, these reads contained only short viral repeating fragments in a long sequence.

The Rapid Barcoding Sequencing kit was the most successful in the identification of pathogens in simulated infection samples.

Keywords: next generation sequencing, Oxford Nanopore, MinION, clinical virology, unknown pathogen, metagenomic sequencing