

Abstract

Next generation sequencing, which allows concurrent parallel sequencing of many samples and makes it possible to distinguish the infection from multiple viral types in the sample, is well suited as a detection format for such assays described below. The aim of the thesis was to develop a method that could detect all known types of human adenoviruses, human enteroviruses, and bacteriophages selected for their presence in the intestine. Using the next-generation sequencing.

The first step was to design primers capable of detecting all known types of viruses, covering the area that is capable of distinguishing these viruses. This method was tested on a set of 47 human adenovirus samples and 30 human enterovirus samples of known serotype. Samples with two serotypes in different proportions were also created. After amplification of the target genome, the samples were purified and sequenced on MiSeq, Illumina. The method was further used in the typing of adenoviruses, enteroviruses and bacteriophages in pre-diabetic cohorts of DIPP, MIDIA, and a cohort of diabetics from African and Asian countries. The tested sample was RNA / DNA isolated from the stool specimen.

We have demonstrated that the method is capable to detect all tested virus types, including infections with two different types, even if the quantity of one of the genotype is up to 1000 times lower than the other. Then was the method tested on MIDIA cohort samples: a total of 7 different adenovirus types were detected, the most common was HAdV2. 26 enterovirus types were found, the most common was CVA2. We also found 19 adenovirus infections of more than one type in a sample and 14 enterovirus infections of more than one type. Bacteriophages were detected and method was tested on samples DIPP cohort and samples of African and Asian diabetics. The most common group of intestinal bacteriophages in these samples was the genus Sk1 virus, followed by the Lambdavirus genus.

The described method was tested and show the ability to detect and distinguish between types of human adenoviruses, enteroviruses or selected bacteriophages.

Key words: adenovirus, enterovirus, bacteriophage, new generation sequencing, autoimmune disease

