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Monitoring of influenza: Whole-Genome Sequencing to provide insights into disease severity

Content

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Every winter, seasonal influenza causes substantial morbidity and mortality and has a significant impact on the economy. Moreover, a new influenza virus subtype can arise and cause a pandemic, with devastating effects on public health, healthcare systems, economy and sometimes also agricultural systems. Therefore, there exists a need for rapid and accurate characterization of the highly dynamic genomes of the influenza viruses for the prevention and mitigation of influenza. High-throughput molecular approaches offer new possibilities for influenza monitoring and global pandemic preparedness. By determining the whole genome of influenza virus, higher resolution evolutionary patterns can be revealed, knowledge of reassortment events and emerging mutations across all genes are provided and information on intra-host diversity of the virus is obtained. This information can lead to a better understanding of genetic changes in all segments during various seasons, possible antiviral resistance, tropism markers, antigenic characteristics, virulence and reassortment events.

We aim to develop a generic whole genome sequencing workflow for influenza A and B viruses to improve the characterization of circulating influenza strains in humans for routine surveillance. Therefore, two published multiplex RT-PCR protocols were improved to generate amplicons of the 8 influenza segments of swab samples, including three universal primers to amplify influenza A segments and a cocktail of 8 primers to amplify B segments. The generated amplicons were used as templates for Illumina MiSeq sequencing. Three viral loads based on the RT-qPCR results of the swab samples, namely high (16 < Ct < 20), moderate (20 < Ct < 30) and low (30 < Ct < 35) Ct of the main human influenza A subtypes, A(H1N1) and A(H3N2), and B were used to assess if all types of influenza can be amplified with the optimized multiplex RT-PCR and to estimate the limit of detection to detect all genome fragments using the whole workflow.

This optimized workflow will be used to sequence a representative subset of 172 samples of the subtype H3N2 from the 2016-2017 influenza season in Belgium corresponding to different degrees of disease severity. This subset will be analysed for specific mutations in the consensus sequence that might be associated with severe influenza cases. These protocols will also be used for the routine surveillance of circulating strains in Belgium. In the future, the quasispecies composition of clinical influenza virus isolates will also be analysed to try to identify a possible relationship between sequence polymorphisms and the reported disease severity.

Choose primary session

Pathogenesis

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Vaccines and antivirals

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