# Development of a real-time PCR assay for quantitative analysis of human influenza A virus replication

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Influenza viruses cause yearly epidemics with considerable impact on health and economics. In pandemic years hundreds of millions of cases occurred throughout the world with a high rate of mortality. To design effective antiviral drugs or vaccines for preventing influenza pandemics a detailed understanding of intracellular processes of host cells during an influenza virus infection is essential. The general course of events during influenza virus replication in host cells are well understood, but much about dynamics and control of expression of the viral genome as well as the transcription of the viral messenger RNAs still remains unclear.

In this work, we apply real-time PCR to investigate the time course of human influenza A virus replication in a transformed MDCK (Madin Darby Canine Kidney) cell line. We designed a two-step technique based on a well-established and frequently used method described for influenza virus detection. In the first step, total cellular RNA was extracted from influenza virus infected MDCK cells or from cell culture supernatant during infection. In a second step complementary DNA was synthesized in a reverse transcription using polarity specific primer: producing complementary DNA of either viral RNA of negative polarity (vRNA(-)) or of complementary RNA and messenger RNA of positive polarity (cRNA(+), vmRNA(+)) for influenza virus. Finally, these transcripts were analysed in a quantitative real-time PCR using SYBR green fluorescence.

Here, we present first results in designing and optimising this sensitive and specific two-step quantitative real-time PCR technique.

The main focus of future investigations will be a comparative analysis with simulation results of a structured mathematical model of influenza virus replication in MDCK cells. It is expected that experimental results clarify basic assumption on the control of viral protein synthesis and viral genome replication.