# RAPID DETECTION OF HUMAN INFLUENZA VIRUSES IN ONE STEP RT-qPCR PORTABLE MICRODEVICE

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**ABSTRACT**

Significant pandemic or epidemic waves caused by Influenza viruses adapted affects to human and animals. This is the cause of great economic losses, million of euros in Europe. Therefore, there is a real need for a test providing early detection and fast typing of the influenza causative agent. Thus it will contribute to the improvement of its treatment and surveillance. Our aim is to develop a rapid and sensitive Point-of-care (POC) diagnostic system to be applied in the field (a school, rest home, farm, remote village, …). It would suppose an enormous benefit for the society. Such a system would also be of huge benefit to the veterinary community, with early detection of avian influenza outbreaks in poultry.

RT-qPCR technology is a fast and sensitive method to detect the presence of influenza genetic material in clinical samples. This work describes an **RT-qPCR microdevice for the detection of influenza viruses** in clinical samples. This portable device is able to perform the retrotranscription (RT) by Real-Time (qPCR) of viral RNA isolated with magnetic beads in a single 3 µl chamber.

## GOAL: EXPERIMENTAL METHODOLOGY AND RT-qPCR DEVICE

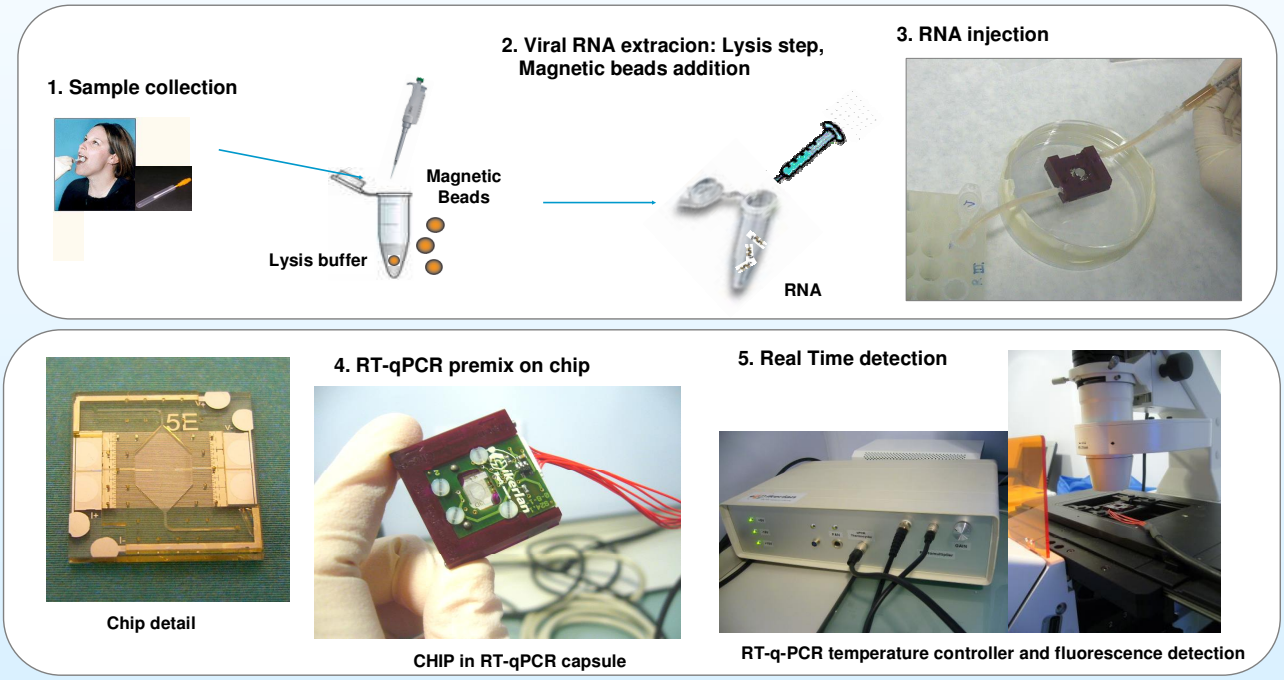


**Figure 1.** Complete flu detection: from real sample to on a chip detection. Procedure for sample collection, target microorganism concentration using immunomagnetic beads, and **ON CHIP RT-q- PCR** for flu virus detection. Nasopharyngeal and throat swabs are collected from patients. Then viral RNA is magnetic beads-based isolated. RT-qPCR mastermix containing RNA isolated from clinical samples is pipetted into the chamber, a chip with electrodes. The chip is then introduced into the capsule that is connected to a small device able to generate the thermocycling steps. The emitted fluorescence signal is captured in real time by a photomultiplier tube through the microchip cover and fluorescence signal is analysed with a data acquisition unit.

## RESULTS: Influenza virus RT- qPCR DETECTION IN CLINICAL SAMPLES

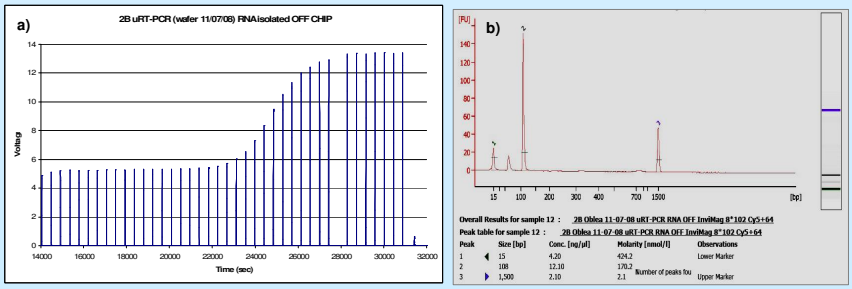


**Figure 4.** RT-qPCR on chip with RNA isolated from clinical samples. **a)** The emitted Cy5 fluorescence signal is captured in real time by a photomultiplier tube through the microchip cover. **b)** After amplification, the reaction mix was collected and the final product was analyzed (Bioanalyzer, Agilent).

## CONCLUSIONS

**We have developed a portable microfluidic device for a specific, rapid and early detection of human influenza viruses in clinical samples (nasopharyngeal and throat swabs). The microdevice is able to perform a one step RT-PCR amplification of influenza molecular markers taking into account the lab on a chip concept. The biochemical reaction is carried out in a 3 µl microchamber, up to 40 minutes.**

**Next experiments will include a new molecular design for influenza typing and an internal amplification control. In a next future we will also include an on chip RNA isolation step directly from clinical samples. Our aim is to provide a more integrated Point of Care microdevice.**

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