Integrated Rotary Genetic Analysis Microsystem for Influenza A Virus Detection

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A variety of influenza A viruses from animal hosts are continuously prevalent throughout the world which cause human epidemics resulting millions of human infections and enormous industrial and economic damages. Thus, early diagnosis of such pathogen is of paramount importance for biomedical examination and public healthcare screening. To approach this issue, here we propose a fully integrated Rotary genetic analysis system, called Rotary Genetic Analyzer, for on-site detection of influenza A viruses with high speed. The Rotary Genetic Analyzer is made up of four parts including a disposable microchip, a servo motor for precise and high rate spinning of the chip, thermal blocks for temperature control, and a miniaturized optical fluorescence detector as shown Fig. 1. A thermal block made from duralumin is integrated with a film heater at the bottom and a resistance temperature detector (RTD) in the middle. For the efficient performance of RT-PCR, three thermal blocks are placed on the Rotary stage and the temperature of each block is corresponded to the thermal cycling, namely 95°C (denature), 58°C (annealing), and 72°C (extension). Rotary RT-PCR was performed to amplify the target gene which was monitored by an optical fluorescent detector above the extension block. A disposable microdevice (10 cm diameter) consists of a solid-phase extraction based sample pretreatment unit, bead chamber, and 4 μL of the PCR chamber as shown Fig. 2.

The microchip is fabricated using a patterned polycarbonate (PC) sheet with 1 mm thickness and a PC film with 130 μm thickness, which layers are thermally bonded at 138°C using acetone vapour. Silica-treated microglass beads with 150∼212 μm diameter are introduced into the sample pretreatment chambers and held in place by weir structure for construction of solid-phase extraction system. Fig. 3 shows strobbed images of sequential loading of three samples. Three samples were loaded into the reservoir simultaneously (Fig. 3A), then the influenza A H3N2 viral RNA sample was loaded at 5000 RPM for 10 sec (Fig. 3B). Washing buffer was followed at 5000 RPM for 5 min (Fig. 3C), and angular frequency was decreased to 100 RPM for siphon priming of PCR cocktail to the channel as shown in Figure 3D. Finally the PCR cocktail was loaded to the bead chamber at 2000 RPM for 10 sec, and then RPM was increased up to 5000 RPM for 1 min to obtain the as much as PCR cocktail containing the RNA template (Fig. 3E). In this system, the wastes from RNA samples and washing buffer were transported to the waste chamber, which is fully filled to the chamber with precise optimization. Then, the PCR cocktail was able to transport to the PCR chamber. Fig. 3F shows the final image of the sample pretreatment. PCR cocktail containing RNA template is successfully isolated from waste. To detect the influenza A H3N2 virus, the purified RNA with PCR cocktail in the PCR chamber was amplified by using performed the RNA capture on the proposed microdevice. The fluorescence images were described in Figure 4A at the 0, 40 cycles. The fluorescence signal (40 cycle) was drastically increased confirming the influenza A H3N2 virus. The real-time profiles were successfully obtained using the optical fluorescence detector as shown in Figure 4B. The Rotary PCR and off-chip PCR were compared with same amount of influenza A H3N2 virus. The Ct value of Rotary PCR was smaller than the off-chip PCR without contamination. The whole process of the sample pretreatment and RT-PCR could be accomplished in 30 min on the fully integrated Rotary Genetic Analyzer system. We have demonstrated a fully integrated and portable Rotary Genetic Analyzer for detection of the gene expression of influenza A virus, which has ‘Sample-in-answer-out’ capability including sample pretreatment, rotary amplification, and optical detection. Target gene amplification was real-time monitored using the integrated Rotary Genetic Analyzer system.

Keywords: Rotary PCR, Rotary sample pretreatment, Integrated microsystem, Influenza A virus