Integrated RT-PCR Microdevice with an Immunochromatographic Strip for Colorimetric Influenza H1N1 virus detection

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Recently, Point-of-care (POC) testing microdevices enable to do the patient monitoring, drug screening, pathogen detection in the outside of hospital. Immunochromatographic strip (ICS) is one of the diagnostic technologies which are widely applied to POC detection. Relatively low cost, simplicity to use, easy interpretations of the diagnostic results and high stability under any circumstances are representative advantages of POC diagnosis. It would provide colorimetric results more conveniently, if the genetic analysis microsystem incorporates the ICS as a detector part. In this work, we develop a reverse transcriptase-polymerase chain reaction (RT-PCR) microfluidic device integrated with a ROSGENE strip for colorimetric influenza H1N1 virus detection. The integrated RT-PCR-ROSGENE device is consist of four functional units which are a pneumatic micropump for sample loading, 2 μL volume RT-PCR chamber for target gene amplification, a resistance temperature detector (RTD) electrode for temperature control, and a ROSGENE strip for target gene detection. The device was fabricated by combining four layers: First wafer is for RTD microfabrication, the second wafer is for PCR chamber at the bottom and micropump channel on the top, the third is the monolithic PDMS, and the fourth is the manifold for micropump operation. The RT-PCR was performed with subtype specific forward and reverse primers which were labeled with Texas-red, serving as a fluorescent hapten. A biotin-dUTP was used to insert biotin moieties in the PCR amplicons, during the RT-PCR. The RT-PCR amplicons were loaded in the sample application area, and they were conjugated with Au NP-labeled hapten-antibody. The test band embedded with streptavidins captures the biotin labeled amplicons and we can see violet colorimetric signals if the target gene was amplified with the control line. The off-chip RT-PCR amplicons of the influenza H1N1 virus were analyzed with a ROSGENE strip in comparison with an agarose gel electrophoresis. The intensities of test line was proportional to the template quantity and the detection sensitivity of the strip was better than that of the agarose gel. The test band of the ROSGENE strip could be observed with only 10 copies of a RNA template by the naked eyes. For the on-chip RT-PCR-ROSGENE experiments, a RT-PCR cocktail was injected into the chamber from the inlet reservoir to the waste outlet by the micro-pump actuation. After filling without bubbles inside the chamber, a RT-PCR thermal cycling was executed for 2 hours with all the microvalves closed to isolate the PCR chamber. After thermal cycling, the RT-PCR product was delivered to the attached ROSGENE strip through the outlet reservoir. After dropping 40 μL of an eluant buffer at the end of the strip, the violet test line was detected as a H1N1 virus indicator, while the negative experiment only revealed a control line and while the positive experiment a control and a test line was appeared.

Keywords: Integrated microdevice, Reverse transcription-polymerase chain reaction, Immunochromatographic strip, Influenza A H1N1 virus, Portable genetic analyzer