SINGLE CHIP INTEGRATED VIRAL RNA EXTRACTION AND RT-PCR FOR INFECTIOUS DISEASE IDENTIFICATION FROM BLOOD SAMPLE
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ABSTRACT
This paper presents an integrated lab-on-a-chip (LOC) microdevice that includes viral ribonucleic acid (RNA) extraction from blood samples and RNA amplification through a one-step reverse transcription-polymerase chain reaction (RT-PCR) method for application on point-of-care (POC) infectious disease diagnostics.

KEYWORDS: Viral RNA Extraction, RT-PCR, Infectious Disease Identification, Blood Sample

INTRODUCTION
Previously, many groups have reported miniaturized nucleic acid (NA) amplification devices such as silicon-based microPCR chip [1,2] or polymer-based micro RT-PCR chip [3], and integrated microdevices for extraction with PCR or RT-PCR [4-7]. However, most of these devices require a skilled operator to apply some form of lab-based sample pre-treatment such as extraction, purification, or separation involving bulky bench-top equipment. As integrated microdevices are designed to process a mixture of say, 100μl of blood sample (equivalent to a few drops of finger-pricked blood) and lysis buffer (150μl of AVL with 200μl of ethanol), the use of such equipment and/or skilled labor can be largely eliminated.

DESIGN AND FABRICATION
Present integrated microdevice consists of three components (Fig.1): 1) microchannel coated with silicon dioxide (SiO2) for solid phase extraction (SPE); 2) spiral-shaped passive micromixer for mixing of eluted viral RNA template into RT-PCR master mix; and 3) thermally isolated meander-shaped RT-PCR chamber having on-chip metallic microheaters and micro temperature sensors. Figures 2 and 3 illustrate the main microfabrication process steps and completed microdevice, respectively. The microchannel area allocated for SPE exceeds 1cm^2 and the volume of the micro RT-PCR chamber is approximately 10μl. We observed the electrical resistances of each microheater and micro temperature sensor to be approximately 110Ω and 650Ω, respectively. The overall chip dimensions are 24×16×1.25mm^3.

Figure 1: Conceptual design of silicon-based integrated lab-on-a-chip microdevice for viral RNA extraction and its RT-PCR amplification.

In order to provide microelectrofluidic interconnections to the microdevice, we custom-made a polycarbonate (PC) packaging (Fig.4) that includes spring-loaded pins (pogo-pins) for electrical interconnections, o-rings with drilled holes having 600μm-diameter for fluidic interconnections and rectangular openings on the top and bottom plates to facilitate thermal isolation of RT-PCR chamber.

EXPERIMENTAL RESULTS
Figure 5 shows an experimental set up with a custom-made temperature controller for integrated sample preparation testing. Figure 6 illustrates a typical controlled temperature profile of RT-PCR chamber during the thermal cycling process.
Table 1. Summarized microbiothermofluidic protocol for the extraction and RT-PCR integrated process on LOC microdevice.

<table>
<thead>
<tr>
<th>Pumping Reagent</th>
<th>Volume</th>
<th>Flow rate</th>
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<tbody>
<tr>
<td>1 Microchannel priming with RNAse free water</td>
<td>30µl/min</td>
<td></td>
</tr>
<tr>
<td>2 Injecting lysed blood sample</td>
<td>500µl</td>
<td>30µl/min</td>
</tr>
<tr>
<td>3 AW1</td>
<td>200µl</td>
<td>50µl/min</td>
</tr>
<tr>
<td>4 AW2</td>
<td>200µl</td>
<td>50µl/min</td>
</tr>
<tr>
<td>5 PCR master mix</td>
<td>100µl</td>
<td>20µl/min</td>
</tr>
<tr>
<td>6 microRT-PCR chamber blocking by Oil plug</td>
<td>50µl each</td>
<td>20µl/min</td>
</tr>
<tr>
<td>7 Thermal cycling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Amplicon ejection by pushing oil</td>
<td></td>
<td>20µl/min</td>
</tr>
</tbody>
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Table 1 shows the main microbiothermofluidic protocol steps, among which the RT-PCR master mix carries dual purposes: 1) elution of viral RNA from SiO2 surface with the master mix’s water component, which is almost identical with elution buffer as well as 2) the original role of a RT-PCR master mix. This resulted in maximum RNA template concentration obtained from the solid phase extraction step. Mineral oil was used to block the microRT-PCR chamber to prevent reagent evaporation (Fig.1). Our LOC microdevice has demonstrated capabilities of viral RNA extraction from a spiked blood sample containing 8pfu of Dengue serotype II (DEN2) virus (Fig.7(a)) and integrated process including SPE and one-step RT-PCR of a spiked blood sample containing 80pfu of Dengue serotype III (DEN3) virus (Fig.7(b)).
Figure 6: Example of the controlled temperature profile of the RT-PCR chamber during the PCR cycling: RT-PCR temperature protocol contains 48°C for 30min of RT step, 93°C for 15min of pre-denaturation, 35 cycles of 15sec of denaturation (93°C), 20sec of annealing (58°C) and 30sec of extension (72°), and finally 72°C for 10min of post extension step, respectively.

Figure 7: Photographs of the gel electrophoresis: (a) extracted from integrated LOC microdevice and amplified by conventional RT-PCR machine (blood sample spiked by 8pfu DEN2 virus); (b) extraction with RT-PCR test result from integrated LOC microdevices (blood sample spiked by 80pfu DEN3 virus).

CONCLUSION
As our LOC microdevice is capable of on-chip integrated extraction and amplification of viral RNA from blood samples, we can conclude that it has the potential for POC applications on infectious disease diagnostics.

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REFERENCES

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