Supplementary Information to paper "A lab-on-a-chip device for rapid identification of avian influenza viral RNA by solid-phase PCR"

The sensitivity of two other AIV identification methods - multiplex solution-based RT-PCR and conventional solid-phase PCR with cover slip, were determined. The limit of detection was compared to that achieved by on-chip solid-phase PCR as described in the paper.

Sensitivity of solution-based multiplex RT-PCR

10-fold serial dilution of AIV strain H16N3 ranging from 10^0 to 10^{-9} was prepared. Hemagglutination (HA) titre values for the AIV strains were measured to be 1:64. The fifty percent egg infectious dose (EID_{50}) calculated for H16N3 strain was $6.7\log_{10}$ EID_{50} per ml. RNA was isolated from the viral dilution series and 5µl of each dilution was used as template. The multiplex RT-PCR was performed using a RT-PCR kit (Qiagen, Hilden, Germany). The RT-PCR conditions were based on the manufacturer's instruction with minor modification. Briefly, a 25 µl reaction mixture contains 5 µl of 5× RT-PCR buffer, 1 µl of 10 mM DNTP mix, 1 µl of enzyme mix, 5 µl of RNA sample, and three pairs of primers, each at a final concentration of 0.2 µM. PCR was carried out in a thermal cycler (MJ Research Inc., MA, USA) and the cycling protocol consisted of 30 min at 50 ºC for reverse transcription, 15 min at 95 ºC for enzyme activation, followed by 40 cycles of 10 sec at 95 °C, 30 sec at 54 °C and 10 sec at 72 °C, finally 5 min at 72 °C for extension. The PCR amplicons were analyzed using slab gel. As shown in Fig. 1, the 100 bp PCR amplicon of M gene was observed up to 10^{-5} or equivalent to 1 $\log_{10}$ EID_{50}/ml.

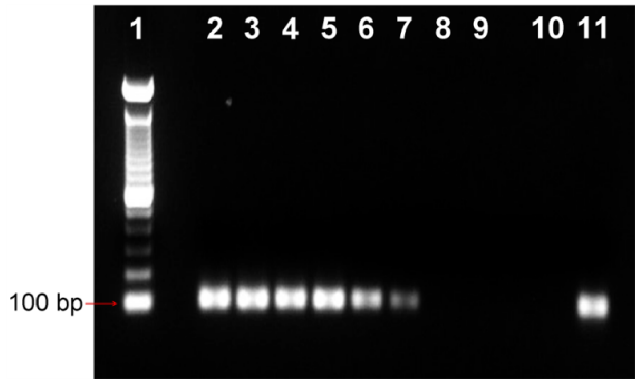

Fig. 1. Detection of H16N3 AIV by solution-based multiplex RT-PCR. Serial 10-fold dilutions of the extracted viral RNA templates, ranging from 10^0 to 10^{-9}, were tested. Amplified products (100 bp target for M gene) with different template concentrations are shown. Lane 1: 100 bp DNA ladder; Lane 2 – 9: 10-fold dilutions of the virus pool ranging from the original HA titre to 10^{-7}, respectively; Lane 10: Negative control (virus free); Lane 11: Positive control. The virus strain was not recognized at the dilutions higher than 10^{-5} or equivalent to 1 log10EID_{50}/ml.

Sensitivity of conventional solid-phase PCR with cover slip

10-fold serial dilution of AIV strain H16N3 ranging from 10^0 to 10^{-9} was prepared and RNA was isolated from the viral dilution series. Microarray was produced on the glass slides by using TC-tagged probes as described in the paper. 25 µl reaction master mix for solid phase RT-PCR contains

10 µl of 5× RT-PCR buffer, 1 µl of 10 mM DNTP mix, 1 µl of enzyme mix, 5 µl of RNA sample, three pairs of primers with each at a final concentration of 1 µM, 2.5 µl of 2.5 µg/µl BSA and 6 µl self sealing reagent (MJ Research, Inc., MA, USA).

10 µl master mix was loaded directly on the oligonucleotide microarrays and cover slips were mounted to seal the reaction droplets. The glass slides were transferred into a twin tower PTC 200 slide thermocycler (MJ Research Inc., MA, USA). PCR was carried out according to the following program: 15 min at 50 ºC for reverse transcription, 10 min at 95 ºC for enzyme activation, followed by 40 cycles of 10 sec at 95 °C, 30 sec at 54 °C and 10 sec at 72 °C, and finally 5 min at 72 °C for extension. After cycling, the slides were washed with agitation in 0.1× SSC/0.1% SDS for 10 min, followed by a short rinse in deionized water. The microarrays were scanned by ScanArray Lite (Packard Bioscience, MA, USA), with appropriate laser power and PMT settings. ScanArray software (Packard Bioscience, MA, USA) was used to quantify the spots by calculating the average pixel intensity inside the defined spots.

As shown in Fig. 2, similar detection limit was obtained for solid-phase RT-PCR with cover slips, where the lowest concentration of template at which positive fluorescence signals could be detected was also 10^{-5} or equivalent to 1 $\log_{10}$ EID_{50}/ml. In this paper, for solid-phase PCR performed in the microchamber, the lowest concentration of template at which positive fluorescence signals could be detected was 10^{-6}, or equivalent to 0.7 $\log_{10}$ EID_{50}/ml. Compared to both solution-based multiplex PCR and solid-phase PCR with cover slips, 10-fold improvement was achieved by on-chip solid-phase PCR.

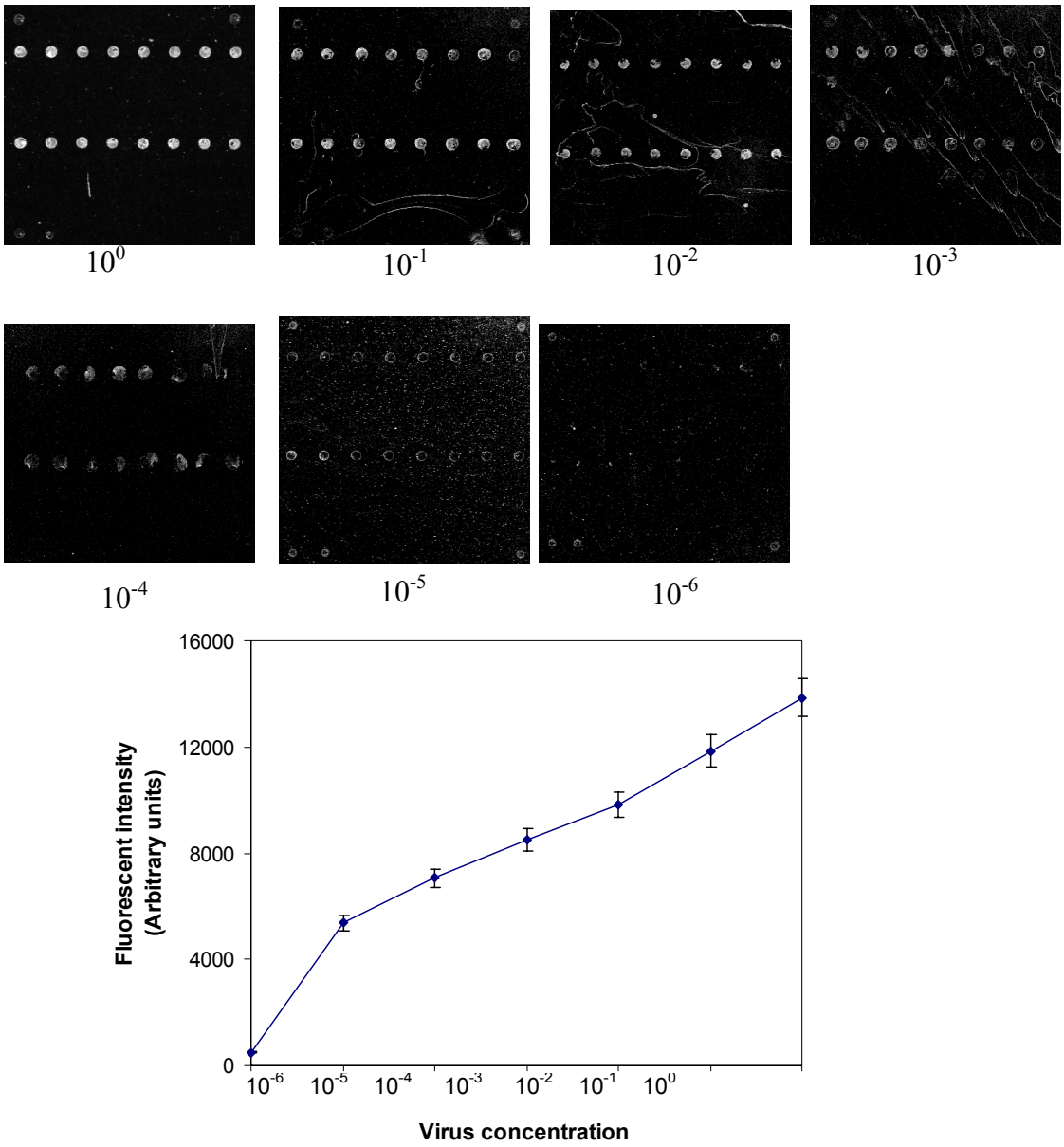

Fig. 2 Detection of H16N3 AIV by conventional solid-phase PCR with cover slip. Serial 10-fold dilutions of the extracted viral RNA templates, ranging from 10^{0} to 10^{-9}, were tested. Fluorescence intensities of probes specific to M gene were measured. The detection limit was also 10^{-5} or equivalent to 1 $\log_{10}$ EID_{50}/ml.