

SUCTION-TYPE MICROFLUIDIC IMMUNOMAGNETIC BEAD-BASED SYSTEM FOR RAPID DETECTION OF INFLUENZA INFECTION

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ABSTRACT

Seasonal and novel influenza infections have caused worldwide pandemic. In order to properly treat the infected patients, a rapid and accurate influenza diagnostic tool should be developed. This study therefore presented a new integrated microfluidic system for detection of influenza infection. It integrated a suction-type, pneumatic-driven microfluidic control module, a magnetic bead-based fluorescent immunoassay (FIA) and an end-point optical detection module. This new system could successfully distinguish the influenza A and B within 15 minutes, respectively. Furthermore, the results from 40 patient specimens have concluded that 75% sensitivity and 75% specificity in both positive and negative detections. This developed system would provide a powerful platform for fast screening of influenza infection.

KEYWORDS: Influenza, Microfluidics, Rapid diagnosis

INTRODUCTION

The seasonal influenza infection is caused by influenza virus. Sometimes influenza may cause serious pandemic infection around the world, such as Hong Kong flu in 1968-1969 which caused about one million deaths worldwide. Furthermore, in 2009, recently, a novel influenza virus containing RNAs from human, pig and avian flu virus has caused serious concerns. In order to cure the influenza infected patients appropriately and immediately, many rapid influenza diagnostic tests were developed, such as QuickVue[®] Influenza A+B Test (Quidel). However, the sensitivity shows only 63% for H1N1 seasonal influenza [1].

The advancement in microfluidics has attracted considerable interests and made substantial impact for the development of compact systems for in-vitro diagnosis devices. For instance, our group have reported an integrated microfluidic system for rapid detection of influenza A virus and successfully combined a three-dimensional (3D) magnetic-bead-based FIA and an optical detection module [2]. However, it can only perform one detection at a time. Furthermore, only Influenza A detection was demonstrated. Based on our previous results, a microfluidic system equipped with four detection regions has been developed in this study. Different specific mAbs and fluorescence dyes for detecting influenza A and B can be successfully realized within 15 minutes. Furthermore, 40 patient specimens have been tested. Experimental data showed that 75% sensitivity and 75% specificity can be achieved.

EXPERIMENTAL

The integrated microfluidic chip was composed of micropumps, microvalves and micromixers. Figure 1(a) shows a schematic illustration of the microfluidic chip, which is composed of four detection regions for positive control, negative control, influenza A and influenza B, respectively. Figure 1(b) shows a photograph of the developed microfluidic chip. The dimensions of the chip were measured to be 6.7 cm x 5.5 cm. The micro mixer allows the magnetic beads surface-coated with antibodies to perform immunological diagnosis [3]. This system showed its specificity by utilizing the specific anti-influenza A or B nucleoprotein (NP) monoclonal antibodies (mAbs) which were obtained from the Microbiology and Immunology Laboratory of NCKU. Furthermore, they were directly conjugated with R-phycoerythrin (PE) or Alexa488 fluorescent dye on the developing antibody. Therefore, the accurate type of influenza virus can be detected optically.

The schematic illustration of the assay used in this study was shown in Figure 2. The magnetic beads coated with anti-influenza NP mAb were first loaded in the reaction chamber, and were incubated with the clinical sample for 5 minutes after the sample was transported to the chamber by using micropumps. The direct-conjugated PE developing Ab was then transported into the detection chamber to incubate with magnetic beads. The optical detection module then detected the optical signal after the incubation of developing Ab and magnetic beads [2].

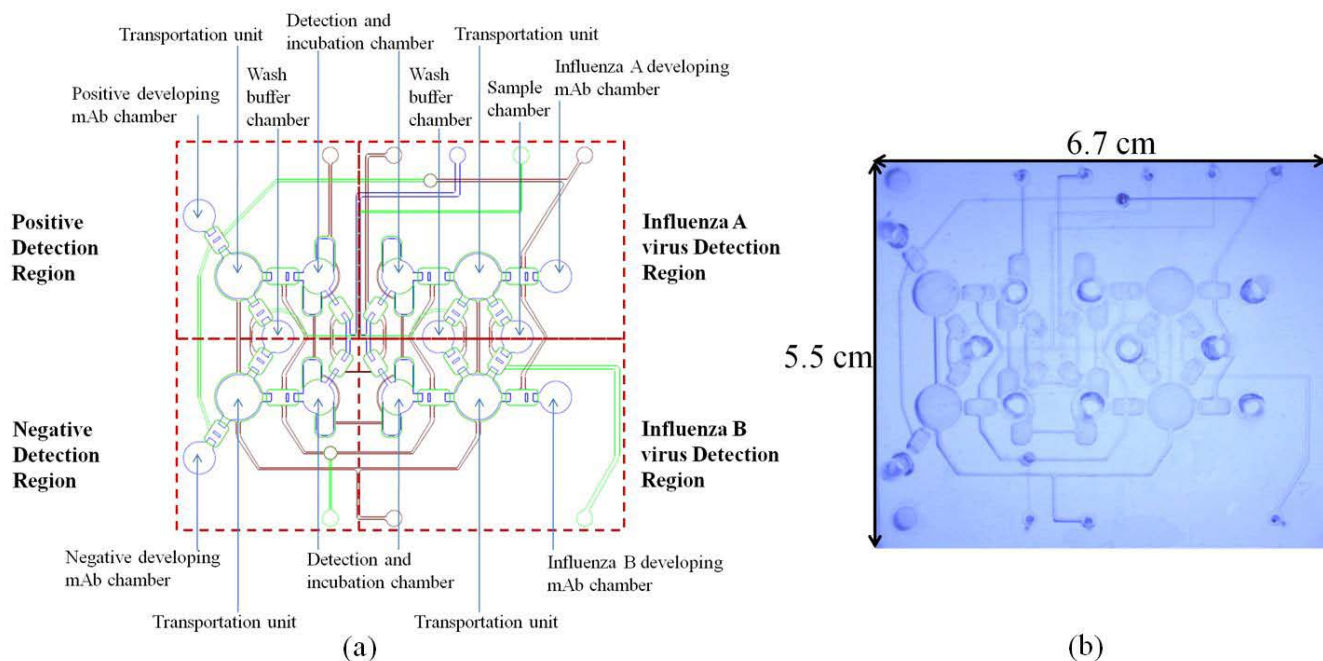


Figure 1: (a) Schematic illustration of the microfluidic chip, (b) A photograph of the microfluidic chip.

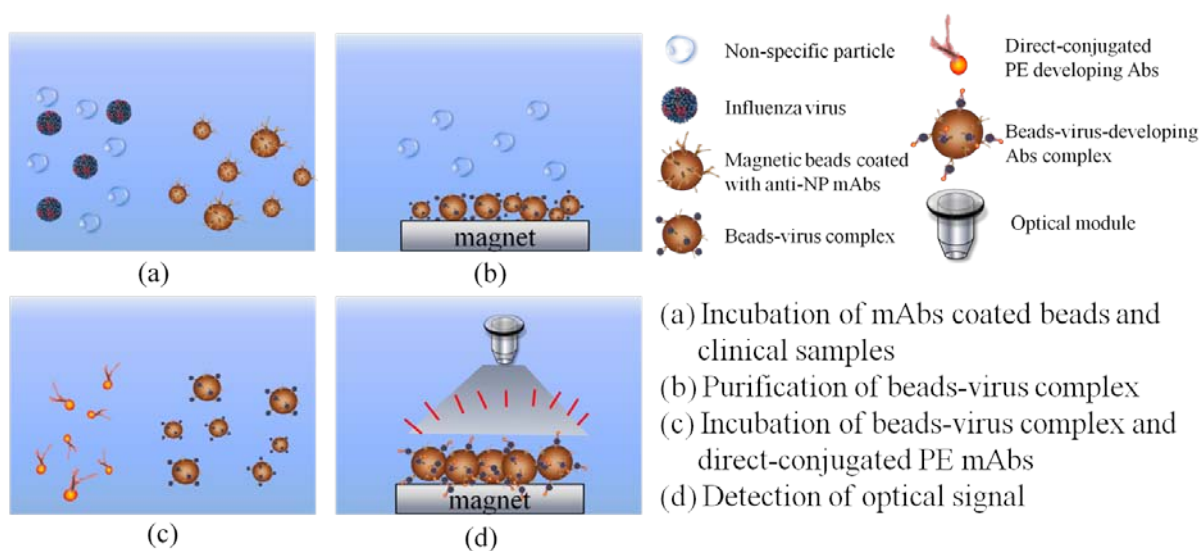


Figure 2: A schematic diagram of the assay used in this study.

RESULTS AND DISCUSSION

Purified viral particles were first used to test our new microfluidic chip. Figure 3(a) shows a series of optical images of the magnetic complexes with different concentrations influenza A virus (InfA). Note that the entire process only takes 15 minutes, which is shorter than the one in our previous work, because direct-conjugated PE mAbs were utilized for one-step process. As the concentration of the InfA increases, the optical signals increase accordingly. Figure 3(b) shows the comparison between the on-chip and manual operation. The limit of detection was measured to be 0.008 HAU when utilizing the microfluidic system, which was more sensitive than the manual operation.

Furthermore, influenza B (InfB) viral particles and new developed mAbs were used. As shown in Figure 3(c), the detection of influenza virus B can be successfully realized. However, it is not as sensitive as InfA since the assay has not yet been optimized. Therefore, the influenza A detection process was used for clinical specimens test. Figure 3(d) shows the test results for on-chip and manual operation. The optical images from the manual operation showed more debris and sputum-like mucus than the on-chip operations. It is because the microfluidic chip can wash out the debris more efficiently and thus reduce the background noise caused by debris or sputum-like mucus. Table 1 shows the summary of the tests. 75% sensitivity and 75% specificity can be verified. The results showed that the developed chip could detect clinical samples successfully.

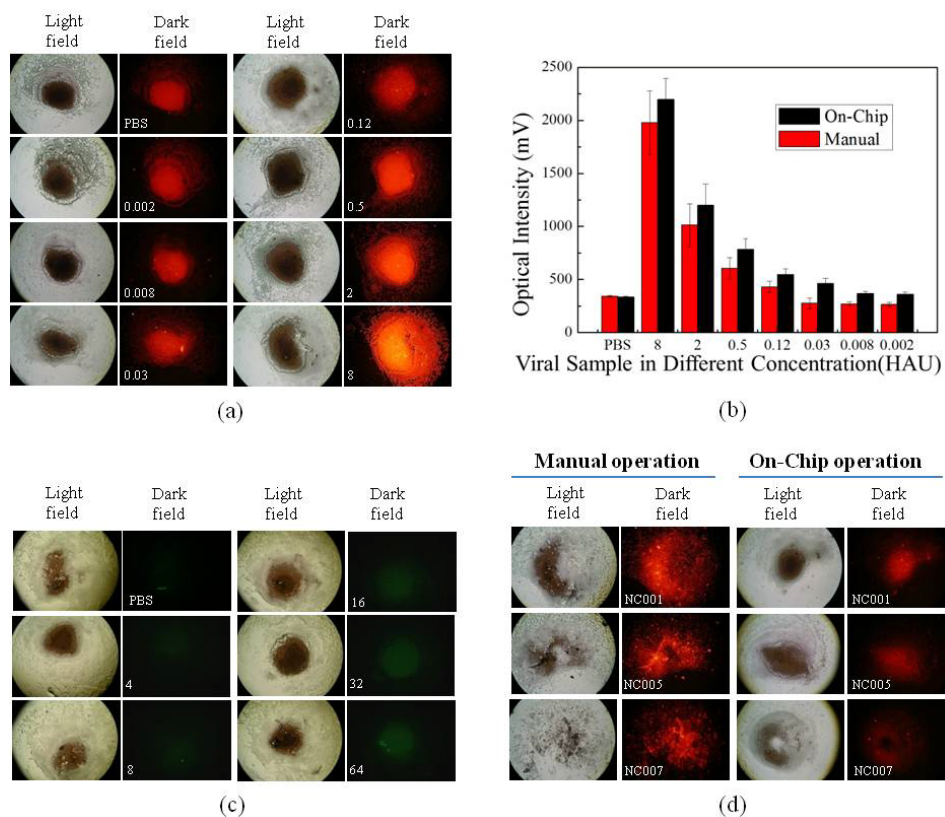


Figure 3: (a) A series of optical images of the magnetic complexes with different concentrations influenza A virus. (b) Comparison between manual and on-chip operation. (c) A series of optical images of the magnetic complexes with different concentrations influenza B virus. (d) Comparison of treatment of clinical specimens between manual and on-chip operation.

Table 1. Two by two table of influenza clinical sample test^a

	Diseased	Non-diseased
Tested positive	15	5
Tested negative	5	15

^a: Sensitivity = 0.75; specificity = 0.75; positive predictive value (pPV) = 0.75; negative predictive value (nPV) = 0.75

CONCLUSION

A new integrated microfluidic system has been demonstrated for diagnosis of influenza infection within 15 minutes by utilizing specific anti-influenza NP mAbs and the direct-conjugated fluorescent developing Abs. Furthermore, 40 patient specimens have been tested with 75% sensitivity and 75% specificity, indicating that this developed platform may provide a powerful tool for rapid detection of influenza infection.

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