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# Integrated On-Chip Microfluidic Immunoassay for Rapid Biomarker Detection

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**Abstract**

Infectious diseases remain a major health concern in many parts of the developing world, where access to adequate health care and modern diagnostic tools are absent. Current diagnostic technologies like ELISA and PCR require large sample volumes, bulky, expensive instrumentation, highly trained personnel, long experimental time, and a modern infrastructure that developing countries lack. Hence, portable, low cost tools would be a huge first step towards making accurate diagnostics available to a wider range of patients worldwide. In this work, we present a portable, microfluidic platform, controlled via a smartphone application, that requires no external pumping and is capable of rapid (within 18 minutes) 6-step colorimetric detection of an array of vaccinia virus proteins spotted on a nitrocellulose pad. We envision this platform as a first step to a fully integrated, portable immunoassay that can be used to expand global healthcare.

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## 1. Introduction

The ability to diagnose infected individuals is the first step to track and prevent the spread of infectious diseases. Due in large part to the availability of modern diagnostic techniques, routine testing and access to quality healthcare, less than 5% of deaths in developed countries are due to infectious diseases. In developing countries, a lack of a modern healthcare infrastructure, electricity, and clean water [1] causes this statistic to jump to 80%. The current state of the art for disease diagnosis includes enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR), both of which are time consuming and require large, expensive equipment operated by highly trained personnel [1]. Many developing countries do not have the resources, infrastructure, or personnel to handle facilities needed to maintain and run such equipment. Thus, biological fluid samples that are collected in remote locations of these countries need to be stored and shipped elsewhere for analysis, where sample degradation and storage conditions are a major limiting factor [1]. With the advent of microfluidic technologies, there has been an increased focus on developing miniaturized, simple, and low power diagnostic tools that can be used in the field to expand access to healthcare globally. Since microfluidics also offers the advantage of using small sample and reagent volumes, cost and assay time are reduced dramatically.

Unfortunately, much of the technology that has been developed to integrate ELISA or PCR into a microfluidic platform still requires bulky, expensive external pumping sources or equipment that can limit portability [2, 3]. Some attempts have been made

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to integrate pumping on-chip but they suffer from inherent challenges to implement in the field. For example, Gao et al. [4] devised a 26 minute assay that used electro-osmosis for sample transportation via pH changes, and fluorescence for detection. Continuous voltage changes are needed for due to different pH of different samples makes the system unreliable for antigen surface binding, and fluorescent detection requires additional expensive equipment. Sista et al. [5] utilized electrowetting to manipulate sample and reagent droplets in the presence an electric field applied via on on-chip micro-electrode array and chemiluminescence for detection. The assay took 7 minutes to complete, but suffered from low throughput in terms of the number of antigens tested per experiment. Micropneumatic pumping by Wang et al. [6], used compressed air and deformable membranes to displace fluid. However, this method required precision control of valve membranes thickness and operation, adding fabrication complexity. Centrifugal force has also been successfully used by Madou et al. [7] for reagent delivery on a CD based platforms, with several commercial products already on the market [8]. Although centrifugal methods are quite developed, the technology requires a rotary system and on disc valves which adds fabrication complexity. Power free transport by Hosokawa et al. [9] used air evacuation and performed the an assay within 20 minutes, but suffered from throughput, once again with respect to number of antigens detected.

Considering the requirement of an efficient pumping system that is easy to fabricate, high throughput, reduced assay time and inexpensive, portable equipment, our group has utilized the LCATs[10, 11] to achieve pumping via cavity-induced microstreaming in a microfluidic platform, negating the need for external pumps. The LCATs can be activated via a small, low power PZT controlled via a smartphone android application. The device is integrated with a nitrocellulose pad that has an array of printed antigen spots specific for a particular disease-causing organism. Serum from a person infected with this particular organism contains antibodies that will bind to these spots. The antibodies are then tagged with a secondary antibody that can provide a visual output (colorimetric) to quantify antibody concentration and patient infection. VCATs were integrated in the device above the nitrocellulose pads to promote mixing and reduce assay time (Fig 1a, b).

**Nomenclature**

LCAT: Lateral Cavity Acoustic Transducers
VCAT: Vertical Cavity Acoustic Transducers
PZT: Piezoelectric Transducer

## 2. Materials and Methods

The microfluidic device was made with Polydimethylsiloxane (PDMS, Sylgard) using standard ultraviolet (UV) photolithographic techniques [12]. Briefly, the two layered SU-8 mold was fabricated by spin-coating SU-8 2050 negative photoresist to a desired thickness, pre-baking, UV exposure for 23sec, post baking the first layer and spin coating followed by pre-baking the resist for second layer. This was then aligned with the second layer mask using a mask-aligner (MA56). This second layer was exposed to UV light for 16sec and the master mold was then post baked and developed using SU-8 developer. The same mold was silanized and kept in a vacuum dessicator overnight. The two components of PDMS, base and curing agent were mixed in the ratio of 11.5:1 and mixed well until the mixture became opaque with bubbles. This was poured on the mold and kept in a vacuum desiccator to remove bubbles. The mold was then placed in a 65$^{o}$C oven to cure overnight and peeled using sharp tweezers once cured. The height of the microchannel was 100µm and the width of the main channel was 500µm. It has been shown that the best efficiency of pumping was given by the LCATs when placed at an angle of 15$^{o}$ relative to main channel [11]. The LCAT side channels were 100µm wide and 500µm long while the VCAT mixers were 100µm in diameter spaced 200µm from center-to-center. The design specifications were chosen for the optimal mixing as demonstrated in previous work [13]. The inlet hole was made with a 4mm punch and the outlet with a 1.5 mm punch. The nitrocellulose pad is 6.5mm long and 6.5mm wide and is placed in a square shaped chamber with two 1.5mm bubble vents punched at the diagonals. The PDMS device is bonded on the glass slide containing the spot bearing nitrocellulose pad using a spray-on hydrophobic coating by Rain-X. Compared to a hexagonal shape, the square shaped chamber is better for relieving air trapped in the device during priming [14].The dimensions of the device are shown in Fig. 1c.

## 3. Results and Discussion

Pumping and fluid transport are major challenges to overcome for portable microfluidic devices. To enable a portable diagnostic device, a pumping mechanism which is easy to fabricate, simple to operate, low cost, and compatible with biological samples is ideal. Our lab has previously demonstrated the coupling of air bubbles with acoustic microstreaming to transfer or mix fluid within a microfluidic device [10]. Acoustic microstreaming uses sound waves to actuate and oscillate an air bubble. The microfluidic chip is placed onto a PZT, which provides the acoustic energy. A function generator energizes the PZT with an alternating square wave. When the square wave is set to the resonant frequency of the air cavity features, the air-liquid interfaces oscillate rapidly and creates eddies in the nearby fluid.

These cavities can produce either pumping or mixing when placed adjacent to a fluid channel. When the cavities are placed at an angle relative to the main channel, a pumping effect is observed (Fig 1c). These angled features are termed as LCATs. When the cavities are placed perpendicular to a fluid channel, mixing is observed in the fluid (Fig 1b). The perpendicular cavity feature