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## Development of a digital microfluidic platform for point of care testing

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### Abstract

Point of care testing is playing an increasingly important role in improving the clinical outcome in health care management. The salient features of a point of care device are quick results, integrated sample preparation and processing, small sample volumes, portability, multifunctionality and low cost. In this paper, we demonstrate some of these salient features utilizing an electrowetting-based Digital Microfluidic platform. We demonstrate the performance of magnetic bead-based immunoassays (cardiac troponin I) on a digital microfluidic cartridge in less than 8 minutes using whole blood samples. Using the same microfluidic cartridge, a 40-cycle real-time polymerase chain reaction was performed within 12 minutes by shuttling a droplet between two thermal zones. We further demonstrate, on the same cartridge, the capability to perform sample preparation for bacterial and fungal infectious disease pathogens (methicillin-resistance *Staphylococcus aureus* and *Candida albicans*) and for human genomic DNA using magnetic beads. In addition to rapid results and integrated sample preparation, electrowetting-based digital microfluidic instruments are highly portable because fluid pumping is performed electronically. All the digital microfluidic chips presented here were fabricated on printed circuit boards utilizing mass production techniques that keep the cost of the chip low. Due to the modularity and scalability afforded by digital microfluidics, multifunctional testing capability, such as combinations within and between immunoassays, DNA amplification, and enzymatic assays, can be brought to the point of care at a relatively low cost because a single chip can be configured in software for different assays required along the path of care.

### INTRODUCTION

Historically, all diagnostic testing was performed at the patient's bedside. While the past century has seen the migration of diagnostic testing to the central laboratory for a variety of reasons, over the past couple of decades, however, some diagnostic tests have begun to shift back to the bedside, paving way for the re-birth of point of care testing (POCT). POCT is defined specifically as "testing at or near the site of patient care"<sup>1</sup> or more generally as "the provision of a test when the result will be used to make a decision and to take appropriate action, which will lead to an improved health outcome"<sup>2</sup>. Under either definition, rapid turnaround time is a key requirement for POCT particularly because it can improve medical decision making. However, rapid turnaround makes a difference between life and death only in certain circumstances and in these cases POCT is well-justified. In other instances, such as in screening for certain types of diseases, rapid turnaround may not have a meaningful impact on therapy, but may still assume great significance in allaying the patient's anxiety. In addition, the rapid availability of results is an increasing expectation in this age of instant messaging.

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POCT was originally applied to blood chemistry, gases, electrolytes, clotting and pregnancy tests. However, with advances in technology, POCT has now been extended to infectious diseases, cardiac markers, and drugs of abuse. The salient features of a point of care device include the following: short time to results (< 15 minutes), ease of use (includes sample preparation and fully automated sample processing), portability (small form factor, if not handheld), small sample volumes (< 30  $\mu$ L requirement allows finger stick rather than venous sample), multifunctionality (capable of combining different types of tests required along the path of care) and low cost. Other important requirements include: self contained reagents, self-calibration and connectivity to laboratory information systems.

Fluid handling is one of the key technical challenges in any point of care device. With the advent of microfluidics, fluid handling has evolved from passive capillarity in absorbent matrices to the use of active fluid handling. The miniaturization brought forth by microfluidics generally allows shorter time to results, integrates sample preparation, and makes fluid handling portable. However, miniaturization alone is not sufficient to make a technology successful in POCT. Other important factors that determine the success of a technology for point of care are the cost of deployment, ease of use and continued maintenance and choice of a problem that requires a point of care device. Though emerging technologies may be capable of providing all the capabilities listed above, there are still significant regulatory issues that will need to be addressed for such technologies to be widely accepted and adopted<sup>3</sup>.

Glucose testing is the most successful and widespread test performed at the point of care and comprises the majority of the POCT market segment. Active fluid handling is typically not required in glucose analyzers since only a single test is performed. In a device that performs more complex testing such as Abbott's i-STAT analyzer<sup>4</sup>, fluids are actively driven through channels to perform testing for electrolytes, metabolites, blood gases, coagulation and more recently, immunoassays. Based on a single POC instrument with application specific cartridges, i-STAT has pioneered the development of automated handheld blood analyzers. While i-STAT has a single cartridge for blood chemistry which combines several tests including metabolites, gases and electrolytes, the coagulation and immunoassay cartridges can only perform one test on each cartridge. Three separate immunoassay cartridges would be required to measure the cardiac markers cTnI, CK-MB, and BNP. Biosite<sup>5</sup>, on the other hand, has a single cartridge which can perform immunoassays on all three cardiac markers simultaneously using lateral flow immunoassay principles. Both of these products are highly successful and have made a huge impact in advancing testing at the point of care.

The current POCT devices are based on a single instrument and different cartridges for different classes of assays. However, in the path of care, a combination of different types of assays might be required on a single cartridge such as, for example, combining immunoassays for cTnI, CK-MB, BNP, along with electrolytes sodium and potassium for cardiac emergency cases or a combination of DNA-based tests and immunoassays for detection of an infectious disease pathogen.

It would be greatly beneficial to integrate immunoassays, chemistry, coagulation and other emerging tests such as molecular testing onto a single platform. Such integration would require advanced fluid handling capabilities beyond simple capillary-driven flow in channels. Most of the current fluid handling approaches are based on continuous flows in channel-based microfluidic systems where a major deficiency is the inflexibility of the fluidic architecture. Most of these devices are designed for specific assay protocols and after the device is fabricated, this paradigm does not easily permit changes in fluidic protocols or between types of assays in real time. The continuity of fluid makes it inherently difficult to operate different areas of the chip independently without using valves. In channel based systems such as the i-STAT and Biosite devices, it would be difficult to multiplex beyond a certain limit using the same

detection reagents due to diffusion and cross contamination between the various products of reactions which are all interconnected through the fluid. Continuous liquid flows in interconnected channels also put a limit on scalability of the number of independent fluid operations and thereby the complexity that can be achieved.

## Digital Microfluidics

There has been growing interest in droplet-based microfluidic systems<sup>6</sup> in recent years as an alternative paradigm to continuous-flow channel-based systems. Several approaches are reported in the literature for manipulating droplets such as electrowetting<sup>7,8,9</sup>, multiphase flows<sup>10</sup>, dielectrophoresis<sup>11</sup>, surface acoustic waves<sup>12</sup>, magnetic methods<sup>13</sup> and thermocapillary effects<sup>14</sup>. A subset of these approaches falls under the category of digital microfluidics wherein several droplets are individually and independently controllable (similar to logic gates in digital microelectronics). The concept of digital microfluidics was originally described and demonstrated in the context of programmable manipulation of discrete droplets using electrowetting<sup>15</sup>.

Droplet-based microfluidic devices based on multiphase flows can be generally categorized as discrete microfluidics since there is no independent control of individual droplets. Droplets in multiphase flow systems are manipulated in channels whereas in digital microfluidics, droplets are manipulated on open, planar surfaces under programmable software control providing real-time control of individual droplets. Although multiphase flows do not have all the advantages of digital microfluidics, because there is no active control of individual droplets, this technique still benefits from the advantages associated with discrete-flow microfluidics such as discrete microreactors, rapid mixing and reduced dead volumes.

Dielectrophoresis uses a spatially non-uniform electric field to manipulate polarizable liquids such as water. In this method, non-uniform electric fields are applied to droplets using an array of electrodes similar to electrowetting electrode structures but the two techniques target different regimes of dielectric behavior of the liquid. Electrowetting is operated using either DC or low frequency AC electric fields where the manipulated liquid behaves as an equipotential body and typically no field is experienced by the liquid mass. Dielectrophoresis is operated typically using high frequency AC fields where the droplet body is subject to electric fields. Dielectrophoresis has been utilized to demonstrate basic droplet operations such as dispensing, transport, and merging<sup>16,17</sup>. A fluorescent protein assay was demonstrated on a dielectrophoresis-based digital microfluidic device as a proof-of-concept for performing biological assays<sup>11</sup>. While dielectrophoresis is becoming a more versatile tool for digital microfluidic manipulation, limitations still exist for handling liquids such as physiological samples, highly conductive culture media and lysis buffers where Joule heating-related issues may impede its development for enabling point of care diagnostics.

Surface acoustic waves (SAW) are another potential technique for enabling digital microfluidics where a high frequency voltage applied to electrodes on a piezoelectric crystal excites a SAW, which can be utilized to manipulate the liquids. Basic digital microfluidic droplet operations such as mixing and transportation as well as biological applications such as PCR and DNA hybridization have been demonstrated using SAW-based microfluidic devices<sup>18,19</sup>. Thermocapillarity is another technique that can enable digital microfluidics, where temperature gradients across a droplet induce thermocapillary stresses that result in droplet motion. Using thermocapillarity-based actuation, the effect of temperature on the enzymatic reaction rate of  $\beta$ -D-galactosidase was measured<sup>20</sup>. Another technique for manipulating individual droplets utilizes magnetic forces acting on magnetic beads in a droplet. Droplet transport, splitting and merging have been demonstrated both with<sup>21</sup> and without a moving magnet<sup>13</sup>. Electrochemical detection of dopamine and glucose was performed using

voltammetry and amperometry respectively on a magnetically actuated digital microfluidic device<sup>22</sup>.

### Digital microfluidics using electrowetting

Although any of the techniques listed above could be utilized for effecting digital microfluidics, currently their enablement for point-of-care applications is minimal. No clinically relevant assays have been demonstrated nor have real physiological samples been shown to be compatible. Using electrowetting, liquids with a wide range of fluid properties (interfacial tension, viscosity, ionic strength and protein content) have been demonstrated to be compatible with digital microfluidic operations such as transporting, mixing, splitting and dispensing. Several biological applications have been demonstrated on electrowetting-based digital microfluidic devices including clinical diagnostics on human physiological fluids<sup>23</sup>, enzymatic assays<sup>24,25</sup> and nucleic acid amplification (PCR)<sup>26,27,28</sup>.

In this paper, we report on the development of immunoassays and DNA amplification on our electrowetting-based digital microfluidics platform with particular emphasis on reducing the time-to-result and integrating sample-to-answer functionality, which are critical for point-of-care devices. Although clinical chemistry is a major segment for POCT, we do not address it in this paper because we have previously demonstrated chemistry assays on our digital microfluidic platform. Other aspects of point-of-care devices such as portability, multifunctionality and cost are also discussed.

### Electrowetting architecture

**Digital microfluidic cartridge:** An electrowetting-based digital microfluidic cartridge consists of a “chip” with electrodes to control the droplets and a conductive “cover plate” which provides the reference potential to the droplet. The chip and the cover plate are arranged in a parallel plate configuration with the droplets sandwiched between them. The droplets are surrounded by an immiscible fluid to prevent evaporation and to facilitate droplet manipulations. The basic construction and operation of an electrowetting device has been described in detail previously<sup>7</sup>.

Figure 1 shows the picture of a fully assembled cartridge on the left with the different components labeled and the instrument on the right. The functional components of the lab-on-a-chip are the fluidic input ports, droplet aliquoting units and droplet pathways for transport, mixing, incubation, washing and detection.

**Fluidic Interface:** The fluidic input port is the interface between the external world and the lab-on-a-chip. The design of the fluidic input port has been a challenge in microfluidic devices due to the discrepancy in the volumes of real world samples (several microliters to 10s of milliliters) and lab-on-a-chip capacity (submicroliter). The digital microfluidic cartridge consists of two types of reservoirs; one where the liquid is stored on-chip between the chip and cover plate and a second off-chip reservoir extending above the plane of the cover plate. For larger input volumes (several  $\mu\text{L}$  to several mL) liquid is simply dropped or pipetted into the off-chip reservoir. The off-chip reservoir is connected to the chip through a hole in the cover plate located above the reservoir electrode.

**Droplet aliquoting:** For both on-chip and off-chip reservoirs, droplets are aliquoted from the reservoirs by switching electrodes proximate to the input port. We have previously described droplet aliquoting from on-chip reservoirs<sup>23</sup>. Aliquoting from off-chip reservoirs is similar and involves the following steps. 1. The reservoir electrode is turned on to prime the on-chip reservoir with the liquid from the off-chip reservoir. 2. A liquid column is extruded from the on-chip reservoir by activating a series of electrodes adjacent to it. 3. Once the liquid column

overlaps the electrode on which the droplet is to be formed, the intermediate electrodes are deactivated to pinch-off a droplet.

**Droplet pathways:** Droplet pathways consist of contiguous unit electrodes, which connect different functional areas of the chip. The area of the unit electrode and the separation between the chip and the cover plate determines the unit droplet volume. These electrodes can be used either simply for transport or for other complex operations such as mixing and splitting. In order to minimize the number of electrical contacts, a multiphase transporter design<sup>29</sup>, where multiple unit electrodes share a single electrical pin, is preferable for the fluidic pathways. In an n-phase transporter, every nth electrode is electrically connected. Mixing of two droplets on the chip is simply achieved by merging them and moving the merged droplet along a linear path. Droplet motion in an electrowetting system is known to create internal circulating flow patterns which enhance the mixing process<sup>30</sup>. Chemical and biochemical reactions (including incubation) can be performed on-chip by merging reacting droplets and mixing them.

**Washing:** Washing is a key process in protocols involving solid phases (typically magnetic beads) such as for example, immunoassays, sample preparation and extraction. During washing, the solid phase is immobilized and unbound molecules can be removed either by serial dilution, which involves repeated addition of a wash buffer droplet and splitting away the excess supernatant until the supernatant is free of unbound molecules or by complete fluid replacement where the beads are completely extracted from the liquid and then resuspended in a fresh wash buffer droplet. In the experiments reported in this paper, we adopted the serial dilution method where we use magnetic beads as the solid phase, which are immobilized using a permanent magnet. Additional details describing the washing protocol are presented in the immunoassay section of this paper. Elution is a subset of washing in which the supernatant is used for downstream analysis.

## EXPERIMENTAL SETUP

### Cartridge design

Both immunoassay and DNA amplification experiments were performed on digital microfluidic multiwell plate cartridges (Figure 1). The cartridge has 12 sample reservoirs and 8 reagent reservoirs. The loading ports for sample and reagent reservoirs are located on a standard SBS 384-well plate format. Sample reservoirs are 4.5mm apart and the reagent reservoirs are 9mm apart. The 384-well plate format for fluidic inputs makes the cartridge compatible with high throughput robotic systems. Off-chip reservoirs to hold large volumes of liquid for wash buffer, waste and reagents required in larger volumes (chemiluminescent substrate) were attached to the digital microfluidic chip.

All whole blood experiments were performed in a smaller format cartridge (3.04 cm × 6.09 cm) which is more suitable for point of care applications. However, most of the experiments described here were performed on a multiwell-size cartridge (8.55 cm × 12.78 cm) in order to allow higher throughput of experiments during development phase.

Figure 2 shows the configuration of the well plate cartridge for immunoassay and PCR application. The key specifications of the cartridges used in the different experiments are listed in Table 1.

### Cartridge manufacturing

A low cost for the consumable (cartridge) is an important consideration for point of care devices. For this reason, we have chosen an inexpensive printed circuit board process<sup>31</sup> to manufacture the digital microfluidic chips. The cover plate is an indium tin oxide coated piece

of glass with loading ports mechanically drilled for fluid input. Both the cover plate and the chip are hydrophobized. A photolithographically patterned polymer film serves as the spacer between the chip and the cover plate and also defines the reservoirs on-chip. The chip is attached to the cover plate using an epoxy. A well plate with off-chip reservoirs is attached to the cover plate.

## Hardware design

**Magnetic setup**—Neodymium magnets (1/8"X1/8"X1/16"-Grade ND 42) were obtained from KJ Magnetics (cat #B 221). The magnets were embedded on the cartridge deck at appropriate locations depending on the application and the protocol used.

**Optical Detection for immunoassays**—We have selected chemiluminescence as a detection mechanism for measuring the bound antibody due to the high sensitivity afforded by the technique. Chemiluminescence measurements were obtained in a plane perpendicular to the digital microfluidic cartridge using a photon counting photo multiplier tube (PMT) obtained from Hamamatsu. The photon counter has an 8mm diameter window for light collection which is coupled to the chip with a custom-made lens to improve the light collection efficiency. The lens was placed at a distance of ~250µm from the top side of the cover plate to maximize the light collected from the droplet.

**Optical detection for PCR**—A miniature fluorimeter, that comprises a light emitting diode and a photodiode, was made in-house and aligned to specific areas on the chip to enable real-time detection for the PCR reaction. The fluorimeter is the size of a match-box and fits easily along side the PMT used for immunoassays within the current instrument shown in Figure 1. The excitation wavelength was 475nm and the emission filter's wavelength was centered around 525nm and it was used along with a long-pass dichroic mirror.

**Heater design**—Two aluminum heater bars were placed underneath the cartridge as part of the cartridge deck for thermal control. Each aluminum heater bar is equipped with a silicone rubber heater (Minco HR5160R44.0L12A) and a thermistor sensor (TS67-170, Oven Industries) connected to a PID module controller (SR7-001, Oven Industries) to achieve temperature control. The power for the heating system is provided by a DC power supply.

**Electrical interface**—The digital microfluidic cartridge is controlled using an electrical controller, which has a microprocessor and switching circuitry to control up to 64 high-voltage electrical pins. Electrical connection between the controller and the cartridge is effected using spring-loaded connector pins aligned to pads on the cartridge. The switching is controlled using custom written software.

## MATERIALS AND METHODS

### Immunoassays

**Reagents**—Immunoassay kits were obtained from Beckman Coulter for Troponin I (TnI) containing capture antibodies conjugated to magnetic beads, reporter antibodies labeled with alkaline phosphatase (ALP) and standards (0 ng/mL-100 ng/mL). Chemiluminescence substrate for ALP (Lumigen APS-5) was obtained from Lumigen Inc. (Southfield, MI, USA). Wash buffer was 0.05M Tris-HCl, 0.1M NaCl, 0.02% Tween 20 and 0.1 mg/mL bovine serum albumin, pH 9.5.

Discarded whole blood samples (obtained from anonymous healthy individuals) were procured from Duke University Medical Center, Durham, USA. TnI standards were prepared by dilution into whole blood at a ratio of 1 part TnI standard: 4 parts blood. The concentrations of the

standards that were used to spike the samples were 5, 25, and 100 ng/mL resulting in final TnI concentrations of 1, 5 and 20 ng/mL in blood.

**Droplet-based Magnetic Bead Immunoassay Protocol**—In our droplet-based magnetic bead immunoassay protocol<sup>32</sup>, a sample droplet is mixed with a droplet containing magnetic beads with primary capture antibodies and another droplet containing the secondary antibody labeled with ALP (reporter antibody) where all the droplets are dispensed from their respective on-chip reservoirs and transported to the reactor zone (shown in Figure 2-left). During incubation, droplets are shuttled, split and merged to improve binding efficiency. After the formation of the capture antibody-antigen-reporter antibody complex, the magnetic beads are immobilized with a magnet while the unbound material is washed away. After a specific number of serial dilution based wash steps, each droplet is transported into a detection loop where a chemiluminescent reagent droplet is dispensed and merged with the bead droplet which produces chemiluminescence from the enzyme-substrate reaction. The chemiluminescent product droplet is then transported to the detection spot and the end point glow of chemiluminescence is detected using the PMT.

The most time consuming steps in an immunoassay are incubation and washing. Time to result is directly affected by the protocols used for incubation, the duration of time for incubating the antibodies and the antigens, and the duration of time for incubating the substrate with sandwich beads, all of which depend on the mixing efficiency within the droplets and the reaction and binding kinetics. The amount of washing required to obtain the required sensitivity can also influence the total time to result for immunoassays.

**On-chip Incubation Protocol**—Incubation protocols on the chip are mainly comprised of merging and mixing several droplets. For magnetic bead based immunoassays, mixing on the chip is a departure from the earlier published work on mixing<sup>33</sup> due to the presence of magnetic beads in the droplet and a magnet in the vicinity. The sequence of droplet operations in the incubation protocol involves shuttling the droplet along a linear path of electrodes with a splitting and merging step inserted between transport cycles. Splitting and merging the droplet ensures that the beads are well distributed within the droplet. Immunoassays were performed on a 300 nL droplet that contained 5 ng/mL TnI as a model assay using two different incubation protocols, among many other possibilities, with one performed on the magnet as shown in Figure 3 (a) and the other off the magnet as shown in Figure 3 (b). The first incubation protocol was performed by shuttling a merged droplet (sample droplet, capture antibody conjugated magnetic beads droplet and ALP-labeled reporter antibody droplet) across a set of seven electrodes (Steps i and ii in Figure 3(a)) followed by a split and merge sequence performed at the center of the magnet (Steps iii, iv and v in Figure 3(a)) so that the beads are equally distributed between the split droplets. Since the droplets were transported at a switching frequency of 1 Hz, it takes 18 seconds for the droplets to complete one cycle of mixing which we refer to as an “incubation cycle”. Several such incubation cycles are repeated to obtain the required incubation time as a multiple of 18 seconds. In the second incubation protocol, the sequence of droplet operations and the number of electrodes used for incubation is the same but the only difference is that it is performed away from the magnet (the nearest the droplet gets to the magnet is two electrode widths - Figure 3(b)(iii). Immunoassays were performed using both the incubation protocols with varying incubation times and a plot of incubation time versus signal was obtained for both the incubation protocols.

**Washing Protocol**—While incubation benefits greatly from higher mixing efficiency, washing benefits from no-mixing conditions in the serial dilution strategy employed in the current system. The wash droplet should only dilute the beads and not the supernatant that is carrying away the unbound molecules. Therefore, it is greatly beneficial for the wash droplet and supernatant not to mix with each other.

Among numerous methods that could be utilized for washing, immunoassays were tested using only two wash protocols to determine the optimum washing protocol required to achieve a total time to result of <15 minutes. Immunoassays were performed on a 300 nL droplet containing 0 ng/mL TnI using either an elongated droplet or a circular droplet. Zero ng/mL TnI was chosen for this study because this sample would have the greatest amount of unbound reporter antibodies that must be removed during washing. A schematic of the washing protocol for both the elongated and circular cases is shown in Figure 4(a) and (b) respectively. The first washing protocol involves merging of the wash droplet with the magnetic bead droplet where the shape of the droplets is circular as shown in Figure 4(b). The circular shape of the droplet is obtained by operating on a 2x (denotes two unit droplets) droplets using only one electrode each. In the second washing protocol, the wash buffer droplet and the magnetic bead droplet are less rounded and more elongated which was achieved by operating on the 2x droplets using two electrodes each. By activating two electrodes, the 2x droplet conformed to the shape of two electrodes as shown in Figure 4(a).

Even though figures 4(a)(vi) and 4(b)(vi) look the same, the effect of washing is different which is depicted in the different colors used. In the multiwell plate configuration, which was used to perform experiments at high throughput, each wash cycle is about 12 seconds. In the small chip configuration (shown in Figure 15), which was used to demonstrate sample preparation results, each wash cycle is about 6 seconds. The time for each wash cycle depends on the distance a wash droplet has to travel from the wash reservoir to the magnetic beads en route to the waste reservoir, transport speed of the droplets, dispensing and disposal rates of the droplets. The magnetic bead droplets were washed with varying number of wash cycles using both the protocols and the chemiluminescence was read with the PMT after adding the chemiluminescence reagent. A plot of number of wash cycles versus the chemiluminescent signal was obtained for both the washing protocols.

**Immunoassay Signal Optimization**—Chemiluminescent kinetic curves were obtained for TnI (100 ng/mL) by incubating the magnetic beads containing the antigen-ALP labeled antibody complex with the chemiluminescent reagent for 4 minutes. A plot of chemiluminescence versus time was obtained to determine the time required to reach the end point.

**Immunoassay on Whole Blood**—Immunoassays were performed on the whole blood samples and standards using the on-magnet incubation protocol and circular droplet-based washing protocol. The standard curve using TnI standards was obtained to calculate the spiked recovery and other performance characteristics of the whole blood immunoassay.

## DNA Amplification and Extraction Materials and Methods

**Reagents**—The real-time PCR mixture contains 0.2mM each of the dNTPs, 1μM each of the primers (Integrated DNA technologies, IA), 1.5mM MgCl<sub>2</sub>, 0.1units/μl Kapa2G polymerase (KapaBiosystems, MA), and 2X EvaGreen dye (Biotium, CA). The genomic DNA of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* was obtained from America Type Culture Collection (ATCC, VA).

The reagents for DNA extraction and purification for the infectious disease pathogens, including lysis buffer, proteinase K, ChargeSwitch® magnetic beads, and purification buffer, are supplied in a ChargeSwitch® gDNA cell kit purchased from Invitrogen Corp. (Carlsbad, CA).

Discarded whole blood samples (obtained from anonymous healthy individuals) were procured from Duke University Medical Center, Durham, USA. DNA extraction kit from Dynal Biotech was used for on-chip human genomic DNA extraction experiments from whole blood. The on-

chip experimental protocol was similar to the bench protocol recommended by the manufacturer.

**On-chip DNA Amplification Protocol**—Digital microfluidics can be used for rapid polymerase chain reaction (PCR) thermocycling by shuttling a droplet through distinct temperature zones on a chip<sup>34</sup>. During the reaction, two different temperature zones, 60°C and 95°C, are created in the cartridge using the heater bars and a 600 nL droplet of PCR mix (sample droplet plus reagent droplet) is transported between the two zones at the transport rate of 25 electrodes/second. The traveling distance is 16 electrodes each way so that the droplet shuttles between the heat zones one-way in about 0.6 seconds. The fluorescence signal of the droplet is detected in the 60°C zone and recorded after each amplification cycle.

The digital microfluidics PCR system was characterized for the amplification of genomic DNA from various pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*. The thermal cycling conditions were 30 seconds preheat at 95°C for initial denaturation followed by 40 cycles of 5 seconds denaturation at 95°C and combined annealing/extension at 60°C for various times.

The speed and efficiency of PCR, was tested by varying the annealing/extension time from 7 to 13 seconds using 400pg MRSA genomic DNA added to 600 nL PCR mix. The sensitivity of the digital microfluidics PCR system was also investigated with PCR titration experiments using genomic DNA from *Candida albicans*. Samples with different initial template concentrations (1, 10, and 100 template copies in the 600 nL PCR mix) were amplified and the products were verified using agarose gel electrophoresis.

#### **Protocol for Large Volume Extraction Off-Chip for Infectious Disease Pathogens**

—The DNA in a large volume of raw physiological sample can be extracted using a few off-chip steps for further analysis on the digital microfluidics cartridge. As a proof of concept, a nasal swab carrying 30,000 heat killed MRSA cells was used as the sample for DNA extraction. First, the nasal swab was placed in 0.5 mL of lysis buffer with 0.2 mg proteinase K and incubated for 5 minutes to release the DNA. Chargeswitch magnetic beads, (12.5 µg) suspended in 68 µL purification buffer were added to the lysis solution and incubated for 1 minute to capture the DNA. The beads were then concentrated and transferred in 15 µL of solution to the sample well on the digital microfluidics cartridge for further processing on-chip.

The on-chip sequence of operations for droplet-based DNA purification and recovery is illustrated in Figure 5. Using a permanent magnet located underneath the cartridge, the beads in the 15 µL sample were collected at the bottom of the well and a single droplet (~300 nL) containing virtually all of the beads from the original sample was then dispensed from the reservoir, effectively concentrating the beads by a factor of nearly 50. The droplet was then transported to a wash station where the beads were magnetically immobilized and washed with 8 droplets of wash fluid (TE buffer, pH 7.0). After washing, the buffer was changed to an elution fluid (TE buffer, pH 8.5) and the droplets containing eluted DNA were collected within another reservoir. The purified DNA was then dispensed from that reservoir and mixed with multiple sets of PCR reagents. Finally, the droplets were transported to the heater zone where flow-through real-time PCR was performed.

**Protocol for On-Chip Extraction of Human Genomic DNA**—Figure 6 illustrates the schematic of the digital microfluidic chip used for extracting DNA from a whole blood sample. The six on-chip reservoirs, each with a capacity of 2 µL, were used for storing and dispensing the different reagents. A typical protocol for DNA extraction on chip involved the following steps: droplets of paramagnetic Dynabeads® DNA Direct Universal from Dynal Biotech (1.05 µm diameter) suspended in lysis buffer were dispensed from an on-chip reservoir and

transported to a specific location on the chip using electrowetting. The beads were held by a permanent magnet placed underneath the chip; droplets of whole blood were dispensed from a reservoir and were mixed with droplets of lysis buffer (containing 10 M NaOH) dispensed from another on-chip reservoir, into the mixing reservoir in the ratio of 1:6 and mixed for 10 s. Mixing was performed by dispensing a droplet and then merging the droplet back into the reservoir. Droplets of the cell lysate were then transported across the DNA capture beads in succession and the supernatant was pinched off while holding the beads. Droplets of wash buffer stored in separate on-chip reservoirs were then used to wash the beads to remove cell debris. Purified genomic DNA captured on the beads was then eluted and collected at the bead collection reservoir. The collected DNA can then be amplified either on the chip as part of an integrated sample-to-answer chip or in a commercial thermocycler for further DNA processing or diagnostic applications.

## RESULTS AND DISCUSSION

### Immunoassays

**Incubation protocol and Incubation time**—Magnetic beads have a tendency to settle down due to gravity even in the absence of magnetic field and form aggregates. In the presence of a strong magnetic field applied with permanent magnets underneath the chip, the beads are attracted to the bottom of the droplet and have a tendency to form aggregates more rapidly. The aggregation of beads effectively reduces the surface area available for binding thereby slowing the reaction kinetics and eventually affecting the sensitivity and the time to result for immunoassays. Hence it is necessary to keep the magnetic beads dispersed within the droplet and avoid formation of aggregates. Based on our previous work on mixing<sup>33</sup>, we shuttle the droplet across several electrodes followed by a merge and split sequence to affect bead resuspension utilizing the recirculation patterns within the droplet which broadly mimics the vortexing action produced on bench scale equipment. The protocols described in the Experimental methods section utilize such a split and merge sequence to resuspend the beads within the droplet. Figure 7 shows the influence of incubation on the magnet and off the magnet on immunoassay performance measured in chemiluminescence. Four immunoassays were performed with incubation of capture antibody beads, ALP-labelled reporter antibodies, and the sample containing antigen on the magnet with incubation times of 72s, 198s, 450s, and 630s and four more immunoassays were performed off the magnet with incubation times of 90s, 180s, 252s, and 324s. It can be observed from Figure 7 that when incubation was performed on the magnet, the time to reach saturation was almost doubled the time to reach saturation when compared to incubation performed off the magnet although the same droplet operations were performed in both the incubation protocols. This can occur because, in the presence of a magnet, the recirculation patterns produced within a droplet would resuspend the beads only in a lateral plane (x-y direction) because the magnet underneath the chip attracts the beads towards the surface. Whereas when incubation is performed a sufficient distance away from the magnet (which we observed to be greater than 2 electrodes), resuspension would be achieved both laterally and vertically because the beads are not affected by the magnetic field. Such resuspension of the beads provides more surface area for binding eventually increasing the rate of reaction. In order to capture at least 50% of the antigen, the time for incubation should be 130 seconds while it could take up to 600 seconds to capture 100% of the antigen. In certain point of care applications, where the size of the chip and thereby the real estate on the chip is restricted, incubation might need to be performed on the magnet which will take about 10 minutes if 100% antigen has to be captured leaving only 5 minutes for all other operations in the time to result budget of 15 minutes. Therefore, in such a case, incubation may be performed only for 5 minutes but still capturing 80% of the antigen. On the other hand, if real estate is not an issue and if a few more electrodes off the magnet could be utilized for incubation, then 100% of the antigen can be captured within 4 minutes. The same effect of off

the magnet incubation could also be obtained by mechanically moving the magnet farther from the chip but that adds another moving component to the instrument thereby increasing the power consumption and price of the instrument.

**Washing protocol and number of wash cycles**—Since washing on chip involves several dilution steps, the time to result can be seriously affected when several wash cycles are required to achieve the desired wash efficiency. Figure 8 shows the chemiluminescence signal obtained from an immunoassay after the droplets are subjected to different numbers of wash cycles and different wash protocols. In both the cases presented herein, incubation was performed using the off-magnet incubation protocol for 3 minutes. Each wash cycle takes about 10 seconds in the slug-based protocol and 14 seconds in the circular droplet protocol. It can be seen from Figure 8, that when washing was performed using slugs of liquid (or elongated droplets as shown in Figure 4(a)) desired wash levels were achieved using fewer wash cycles when compared to washing using circular shaped droplets. In the former, mixing was minimized and the bulk of the unbound material from the supernatant was replaced with fresh wash buffer, whereas in the latter mixing was ensured by operating the 2x droplets using only one electrode each. The color schemes used in Figure 4(a) and (b) depict the situation achieved by operating the 2x droplet using two electrodes and one electrode respectively. Also, it was observed visually that the dispersion of magnetic beads in the lateral plane was found to be higher in the slug based washing when the fresh wash buffer droplet merged with the magnetic bead droplet. This would enable any unbound antibody trapped in the interstices to diffuse into the supernatant and be washed away in the subsequent washes. Hence desired wash levels were achieved in ~10 washes using slug based washing when compared to >18 washes in the other case. The washing behavior has two distinct regimes, one regime where washing is very pronounced and the second where the washing is subtle. In the slug based washing case, the washing is pronounced with each wash cycle up to 9 cycles and after that the effect of washing is almost negligible. In the circular droplet protocol, the washing effect is pronounced until the 15<sup>th</sup> wash, although the step wash efficiency is less than that observed for the slug-based protocol. Washing is only marginally effective for the circular droplet protocol between 15<sup>th</sup> and 18<sup>th</sup>. This could happen because all the free unbound material may be washed away in the first few cycles and after that, washing only removes the unbound material trapped between the beads. This could be improved by just inserting a few transport cycles between washes to resuspend the beads between the wash cycles so that during the later stages of washing, the unbound material from the interstices of bead aggregates can be effectively removed. Depending on the sensitivity of the assay required and the time to result requirement, the appropriate number of wash cycles can be chosen for the point of care application.

**Incubation of magnetic beads with chemiluminescent substrate**—Another parameter which influences the time to result is the generation of signal during the incubation of the chemiluminescent substrate with the washed magnetic beads containing the antigen-antibody complex. Immunoassays were performed on TnI (100 ng/mL) using the on-magnet incubation protocol and circular shaped washing protocol. Figure 9 shows the kinetics of the reaction between the chemiluminescent substrate and the ALP on magnetic beads for TnI (100 ng/mL) where 90% of the end point signal was obtained in ~120-130 seconds. For a lower concentration of the analyte, maximum signal was achieved in <120 seconds. Therefore, in the current scheme with the type of substrate chosen, 2 minutes was selected as the optimum incubation time to generate maximum signal for the chemiluminescence reaction. However, if the reaction is observed to behave as a flash signal instead of a glow reaction, then the 2 minute incubation reaction time can be reduced to a few seconds.

**Rapid Immunoassays**—Using the optimized protocols for incubation and washing, a full immunoassay was performed on TnI (5 ng/mL). Magnetic beads were incubated with capture

antibody, analyte and the secondary antibody labeled with ALP using the off-magnet incubation protocol. Ten slug based washes were performed to remove the unbound material from the supernatant (~2 minutes). The droplet with washed magnetic beads with the antigen-antibody complex was mixed with one droplet of the chemiluminescent substrate, incubated for 2 minutes and the end point chemiluminescence was detected using a photon counter. In this protocol, the total time to result was ~10 minutes per immunoassay.

**Whole blood Immunoassays**—A POCT device should include sample-to-result functionality for it to be practically useful. Since whole blood is the most commonly analyzed human physiological fluid, the POCT platform should be compatible with it. Immunoassays generally do not require sample preparation since whole blood could be used directly. After the capture event all the unbound material, including cells, could be washed away.

Figure 10(a) shows the sequence of operations in dispensing a droplet of blood from an on-chip reservoir where a column of liquid was extended and split off using electrowetting to dispense a droplet. It should be remembered that the blood sample is surrounded by immiscible silicone oil which enables all the droplet operations. In the absence of oil, the whole blood droplet sticks to the hydrophobic surfaces of the chip and the cover plate and any manipulation of the liquid becomes extremely difficult because the proteins in whole blood adsorb irreversibly to the surfaces. Figure 10(b) shows the on-magnet incubation protocol, which also demonstrates basic droplet operations such as transport, splitting, and mixing, of a 1x blood droplet with a 2x droplet containing magnetic beads with the capture antibody and the secondary antibody labeled with ALP. After incubation, the combined blood and magnetic beads droplets were washed using the circular droplet-based wash protocol described in the previous section. This is the first demonstration of a whole blood immunoassay on a digital microfluidic platform so further work is required to understand the effect of hematocrit, as well as several other sample parameters, on the performance of the assay.

Three 6-point standard curves were obtained by performing immunoassays on TnI standards (0-100 ng/mL) on different chips. The mean of the data were fitted into a single standard curve. A direct comparison between this standard curve with the values obtained from whole blood was performed to evaluate spiked recovery. The percentage recoveries obtained for 1, 5 and 20 ng/mL of TnI in whole blood were 108%, 87% and 77% respectively. From the recovery data, it can be noted that immunochemical behavior on chip was not significantly different for either matrices (buffer and whole blood), even though the responses were slightly lower for higher concentrations of TnI in whole blood, which may be attributed to the presence of alkaline phosphatase inhibitors in blood.

## DNA Amplification and Sample preparation

**Rapid DNA Amplification**—In a POCT device that performs DNA amplification, it is critical to minimize the PCR cycle time. Theoretically, DNA denaturation and primer annealing are almost instantaneous (< 2 sec), while the extension step has a limited speed (usually 50 - 100 bp/sec). The minimum denaturation time is 5 sec for the current digital microfluidic PCR system due to hardware limitations. The real-time fluorescence signals of MRSA PCR on chip with 5 sec denaturation and various annealing/extension times are illustrated in Figure 11.

The real-time signal indicates that 10 sec annealing/extension is adequate to obtain exponential amplification and good PCR efficiency. Further increase in the annealing/extension time only slightly improves the efficiency. The 10 sec cycle time allows a 40-cycle real-time PCR to be completed within 12 min for a 300-400 bp template on the digital microfluidics platform, while a 40-cycle PCR usually requires 1hr or longer on conventional thermal cyclers. Such a significant time reduction is attributed to the fact that flow-through PCR requires no system

temperature cycling and the bulk temperature of the small PCR droplet can rapidly reach equilibrium with the environment when transported between different temperature zones.

Depending on the specific applications, the PCR time can be further reduced with some loss of efficiency. Figure 11 shows that the DNA template was still amplified to detectable levels with 7 sec annealing/extension time, which is enough to provide a simple “yes or no” answer for the presence of a target DNA in a sample. We can therefore envision a rapid PCR of 6 min or less with a simple upgrade to the current digital microfluidics hardware.

The sensitivity of the PCR system was examined with a PCR titration experiment on genomic DNA from *Candida albicans*. There are approximately 80 copies of the target sequence per *Candida* genome. Figure 12 shows the real-time PCR amplification of samples with different initial template concentrations and the corresponding gel images. The result shows that a 10-fold reduction in the number of template copies causes an increase of ~3.3 in the threshold cycle number ( $C_T$ ), which is in good agreement with the theoretical prediction for exponential amplification. The sample with one copy of *Candida* genome was successfully amplified and detected. The digital microfluidics platform has demonstrated capability to perform fast and sensitive real-time PCR and can serve as a useful tool for infectious pathogen detection.

**Sample preparation for DNA amplification**—When analyzing human genomic DNA, only a small volume of whole blood would have to be processed whereas for infectious pathogen detection, larger volumes of sample will need to be processed to extract the pathogen cells or its DNA from whole blood or other samples.

**Large Volume Extraction Off-Chip for Infectious Disease Pathogens**—Pathogen extraction typically requires processing of a large volume of physiological fluid. While several milliliters of physiological fluids can be entirely processed on chip, in consideration of minimizing the time to results, we have adopted a combination approach where some steps of sample preparation are included in the sample collection method off-chip while most of the processing is still performed on the chip. The sample containing DNA was pre-concentrated from 500  $\mu$ L into 15  $\mu$ L and the entire sample was finally collected in a 300 nL droplet which was subjected to PCR. To verify the presence and quality of the sample DNA, a 40-cycle real-time PCR program was conducted on the purified DNA and the result is shown in Figure 13. The positive amplification demonstrated successful DNA extraction and purification from MRSA cells using the sample preparation procedure based on the digital microfluidics platform.

**On-Chip Extraction of Human Genomic DNA**—The extraction of DNA from a raw physiological sample matrix, such as whole blood, is a critical step for any point of care DNA analysis device. Whole blood has a very high load of cells and therefore a large amount of sample is generally not required for human genomic DNA-based testing. We successfully performed DNA extraction from whole blood on-chip following the protocol described in the methods section. In order to verify and quantify the performance of our on-chip DNA extraction method, we performed PCR on a standard bench-based thermocycler (BioRad iQ5) and compared it with both a human DNA standard and a similar DNA extraction protocol performed on blood on the bench (as shown in Figure 14). The following three samples were used: 1. A pure human genomic DNA sample was used as a positive control and amplified on the bench using a 1  $\mu$ L sample of 10 ng/mL human DNA. 2. A 30  $\mu$ L human blood sample was subject to the extraction protocol described earlier and extracted into a final volume of 1  $\mu$ L and amplified on an iQ5 thermocycler. 3. A 300 nL droplet of human blood was extracted into a final volume of 300 nL on-chip and amplified on the iQ5 thermocycler. The results show cycle threshold ( $C_T$ ) values for the positive, bench extracted, and chip extracted samples as 21, 23.3, and 28.8 respectively. The initial sample volume on the bench (30  $\mu$ L) is approximately 2

orders of magnitude higher than that used on the chip (300 nL) which should, under ideal conditions, result in an increase of approximately 6.6 in the  $C_t$  value. The observed shift in  $C_t$  between the chip-extracted and bench-extracted DNA is 5.5 and this could be due to several non-idealities. Besides the low-volume sample handling and complete automation, the main advantage of the on-chip DNA extraction lies in its potential to be integrated with downstream DNA-analysis methods like amplification, splicing, and sequencing. While we have successfully demonstrated on-chip PCR on a variety of different DNA samples on the digital microfluidic chip, efforts are currently underway to integrate on-chip human genomic DNA extraction and on-chip PCR of the same.

### Other Salient Features for a POCT Device

**Portability**—Portability is an important consideration for point-of-care testing. Besides the chip itself, all of the required supporting instrumentation should be compact, robust and inexpensive to allow routine use at the point-of-care. Portability also generally entails usage of small sample volumes. A small sample volume, particularly in home testing, improves compliance with testing because it is less painful to collect. Digital microfluidics is particularly well-suited for this purpose because direct electrical control of fluids avoids the need for bulky and expensive mechanical pumps and valves. Additionally, fluidic connections between the chip and instrument are avoided resulting in greater reliability and ease-of-use. Only electrical connections between the chip and switching circuitry inside the instrument are required to perform the fluid manipulations. Figure 15 shows a picture of our prototype hand held instrument and its corresponding digital microfluidic cartridge. The handheld instrument has a touch screen to choose pre-programmed assays, a USB interface to download new assay protocols, an integrated PMT and all control electronics for autonomous operation. The digital microfluidic cartridge is 3.04 cm × 6.09 cm and was used in the whole blood experiments (immunoassay and PCR) presented in this paper.

**Configurability and Cost**—A point-of-care testing platform should be capable of addressing a wide range of assays, formats, test menus and sample types. The flexibility and programmability of digital microfluidics provides a high degree of configurability to meet these demands<sup>35</sup>. In our digital microfluidic platform individual droplet manipulation is controlled in software, allowing assay protocols to be easily modified or updated without modification of the chip. For example, incubation times, reagent additions, mixing ratios, wash cycles and many other parameters can be controlled through software. The modularity and scalability of digital microfluidics also enables design reuse avoiding long design cycles. New designs can be quickly and reliably generated from an existing library of design elements. Elements such as dispensers, mixers, and transport pathways can be combined using simple design rules to create highly complex microfluidic systems.

In our digital microfluidic platform, a single sample can be divided into multiple droplets which are analyzed separately for different targets. Multiplexing “in space” avoids limitations associated with chemical cross-reactivity and spectral overlap, although some sensitivity may be sacrificed in splitting the sample. Even where reactions are performed separately they can be sequentially routed through a single detection location minimizing the cost and complexity of the detector hardware.

Multiplexing can also be extended to include multiple assay formats since different formats can be implemented on a common platform using a common set of basic instructions. For example, we have demonstrated here that both immunoassays and PCR assays could be performed using the same chip design with some customization of the hardware for each application. Ultimately, we envision a single unified hardware platform suitable for immunoassays, PCR, enzymatic assays, and other applications as well. The capabilities of this

unified platform could be quite broad, encompassing many common laboratory procedures such as pipetting, mixing, bead washing, thermal control/cycling and various optical detection capabilities.

Digital microfluidics potentially enables a wide range of testing capability to be brought to the POC at relatively low cost. Because a single chip design can be configured in software for different applications, manufacturing economies of scale can be realized for the disposable component of the system. Additionally, the development time and cost of new products is minimized because of the design modularity and scalability of these systems.

## CONCLUSIONS

The adoption and success of POCT ultimately depends on a host of factors including clinical, technical, economic and operational considerations. Until now one of the key technical barriers has been the inability to replicate the quality and breadth of central lab testing in a format suitable for use at the point of care. In particular, the automation of fluid handling required to perform sample preparation and complex multi-step test protocols has been difficult to achieve in a format suitable for POCT. Digital microfluidics based on electrowetting provides sophisticated liquid handling capabilities in a portable and economical platform. Immunoassays, PCR, clinical chemistry and sample preparation have all been implemented on this platform using a common library of design elements. In this paper we have demonstrated rapid immunoassays and PCR using our digital microfluidic platform.

An on-chip incubation protocol was developed for immunoassays which reduced the incubation time from 600 seconds (on-magnet incubation) to 240 seconds (off-magnet incubation). Washing protocols were also optimized to achieve near 100% washing in 10 wash cycles. Using the optimized incubation and washing protocols a rapid immunoassay for cardiac troponin I was performed in less than 8 minutes. We have also demonstrated an immunoassay for troponin I on whole blood using the same platform. Using the same microfluidic chip, we have also demonstrated a 40-cycle real-time PCR within 12 minutes by shuttling a droplet between two thermal zones. A number of annealing/extension times were explored and we found that a 10 sec annealing/extension was adequate to obtain exponential amplification and good PCR efficiency. Further increasing the annealing/extension time only slightly improved the efficiency. We further demonstrated the capability to perform sample preparation for infectious disease pathogens (methicillin-resistance *Staphylococcus aureus* and *Candida albicans*) and for human genomic DNA from whole blood using magnetic beads on the same microfluidic chip.

The flexibility and breadth of digital microfluidics in combination with its cost effectiveness and portability make it an ideal platform to address a wide range of problems in POCT. Key development issues in the future include reagent storage on-cartridge, self-calibration and connectivity to laboratory information systems.

## Acknowledgements

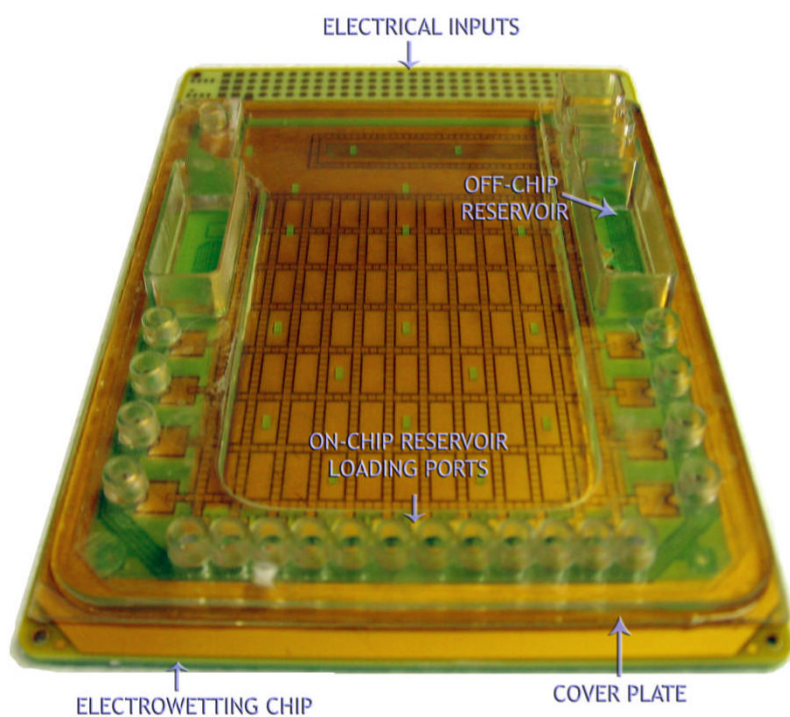
The authors acknowledge contributions from Ryan Sturmer and Greg Smith of Advanced Liquid Logic. The authors also acknowledge funding from NIDDK, NIGMS, NCI, and NIAID (National Institutes of Health, Bethesda, MD, USA) for funding various aspects of the research presented.

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**Figure 1. (Left) A fully assembled digital microfluidic multiwell plate cartridge (8.55 cm × 12.78 cm); (Right) the control instrument (20.32 cm W × 33.02 cm H × 53.34 cm L)**

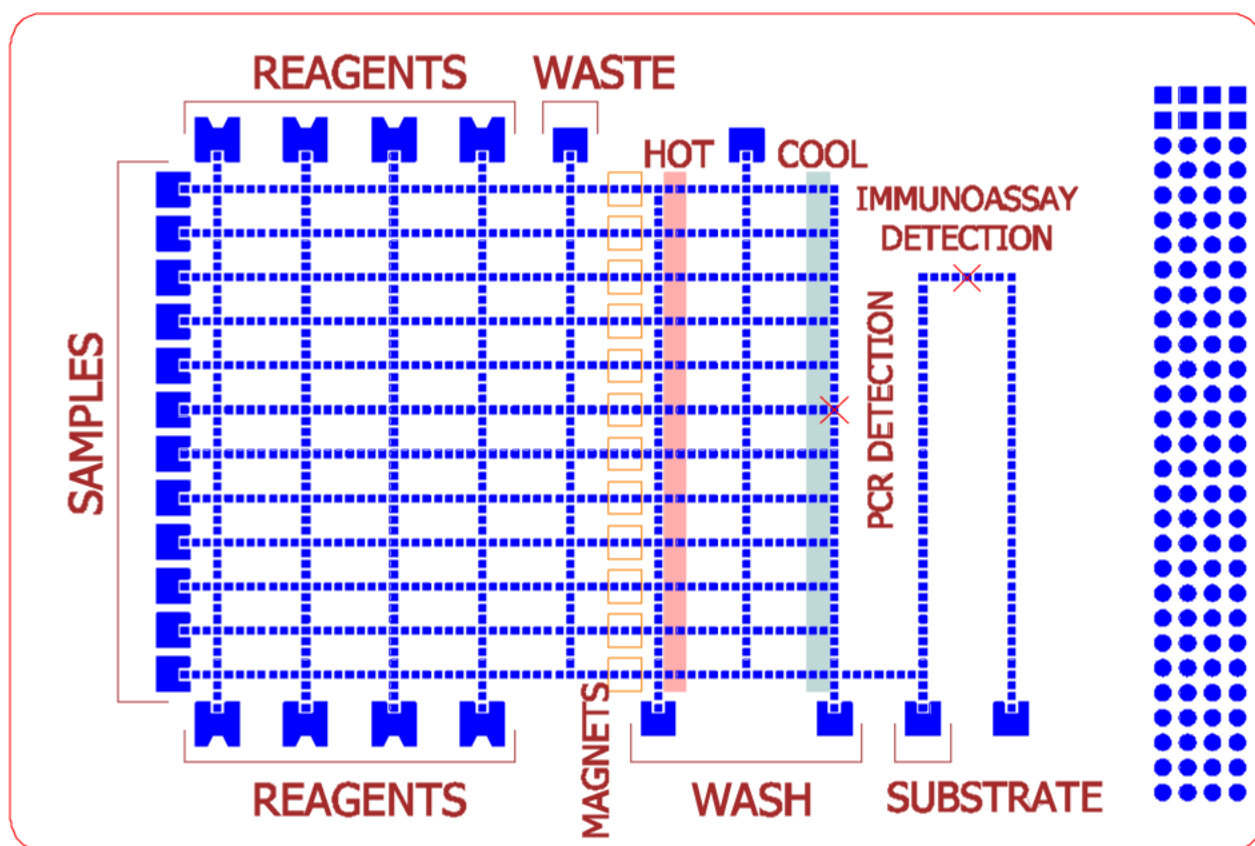
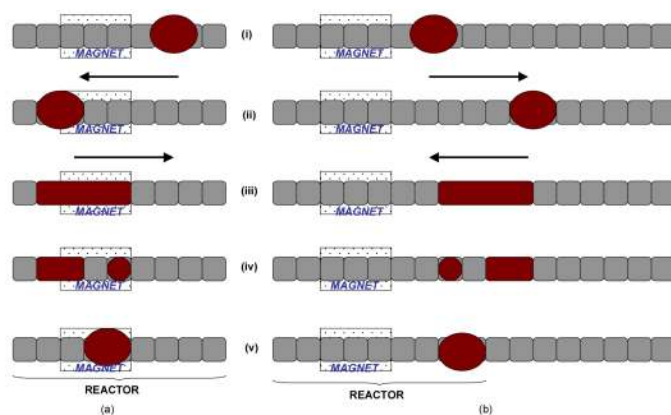
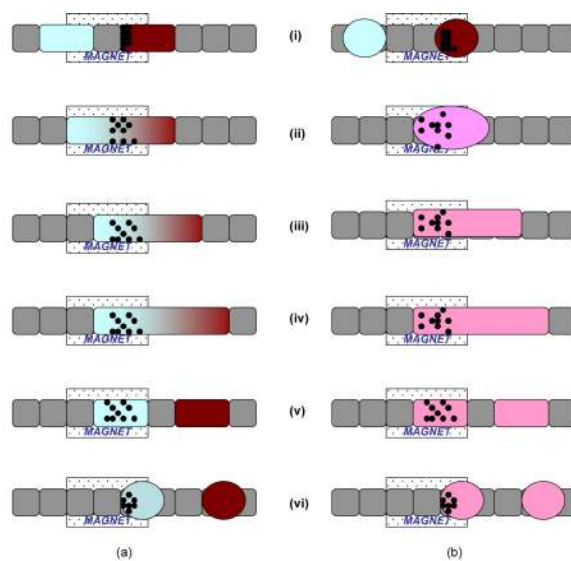


Figure 2. Schematic of the digital microfluidic chip used for immunoassays and PCR



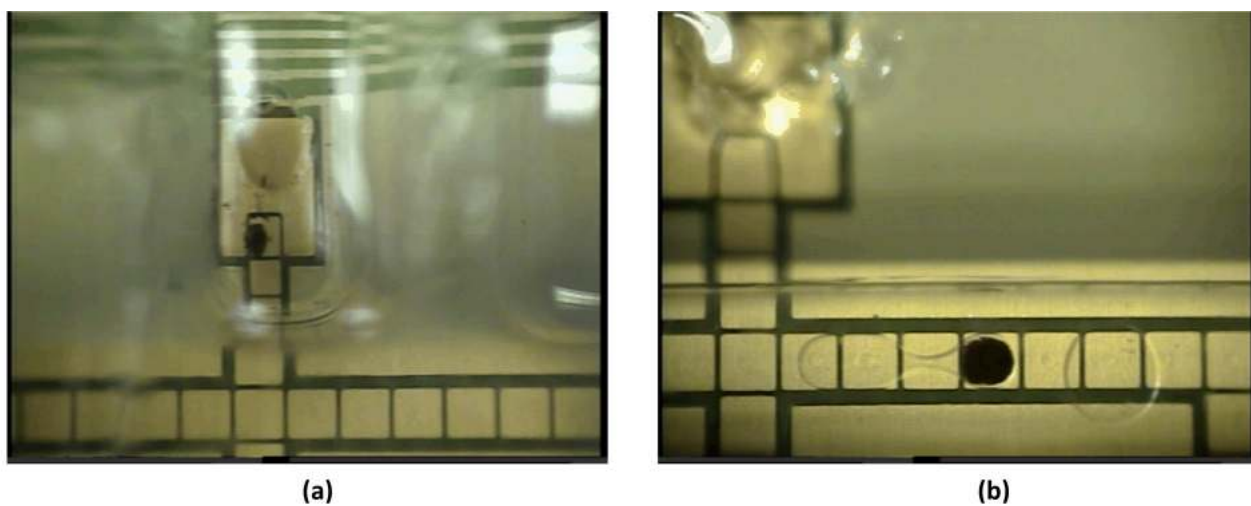
(i)-Merge the droplets (ii)-Shuttle the large droplet (iii) Extend the droplet to form a column (iv) Split the droplet into two asymmetric daughter droplets (v) Merge the droplets and repeat the sequence

Figure 3. (a)- Incubation on magnet, (b)-Incubation off magnet



(i) Fresh wash buffer droplet (ii) Bead droplet merged with fresh wash buffer droplet (iii) Extend the column of liquid (iv) Extend the column more to enable a split (v) Split off the supernatant (vi) Concentrate the beads

**Figure 4. (a)- Elongated droplet washing protocol, (b)- Circular droplet washing protocol**



**Figure 5. Sequence of droplet operations for concentration, extraction, and processing of DNA from a large sample volume containing infectious disease pathogens. (a) concentration of DNA-carrying magnetic beads in the sample well (b) washing and elution of DNA from the beads over a magnet**

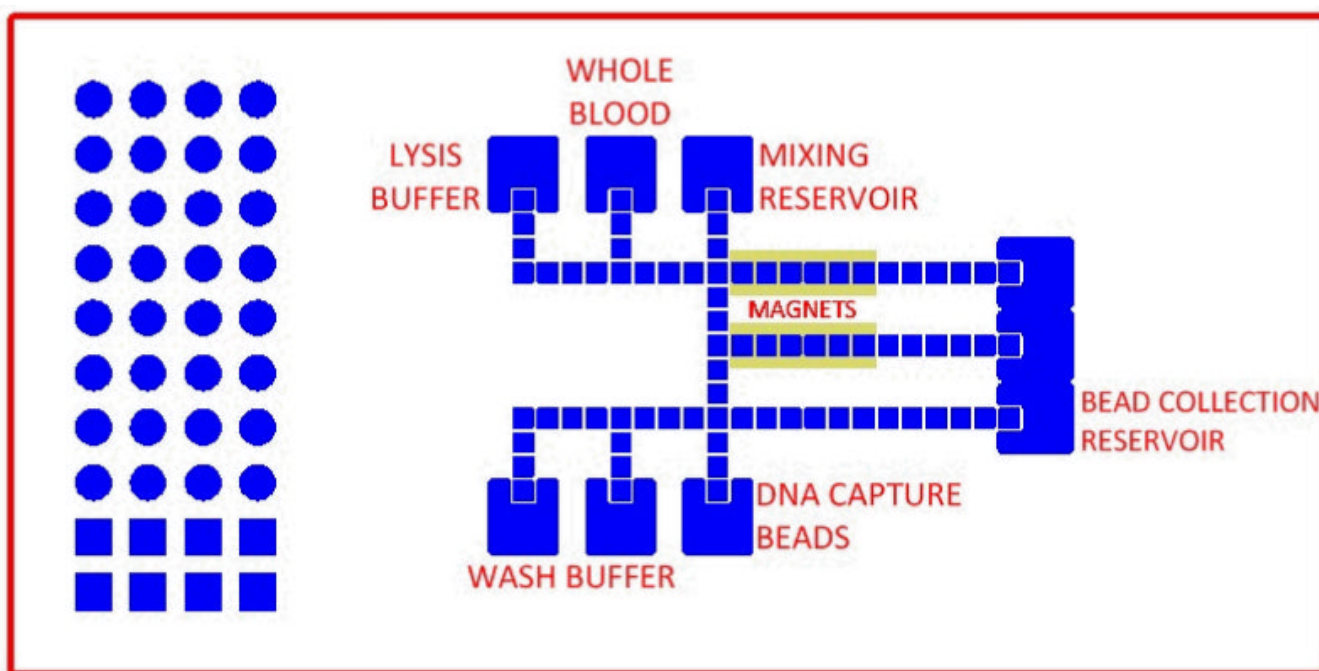


Figure 6. Schematic of the chip used for extraction of human genomic DNA from whole blood

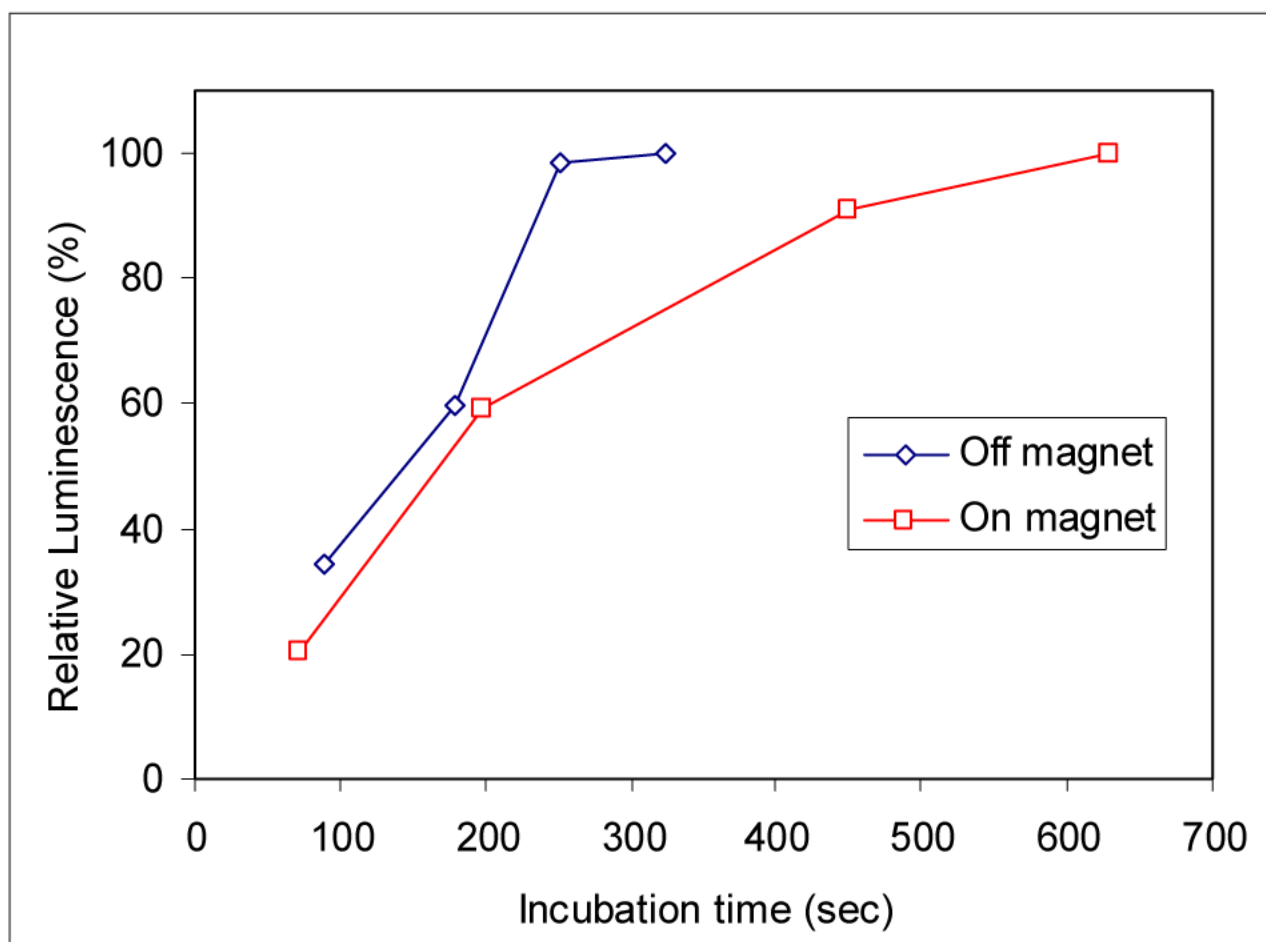


Figure 7. Comparison of incubation time and incubation protocol between on-magnet and off-magnet

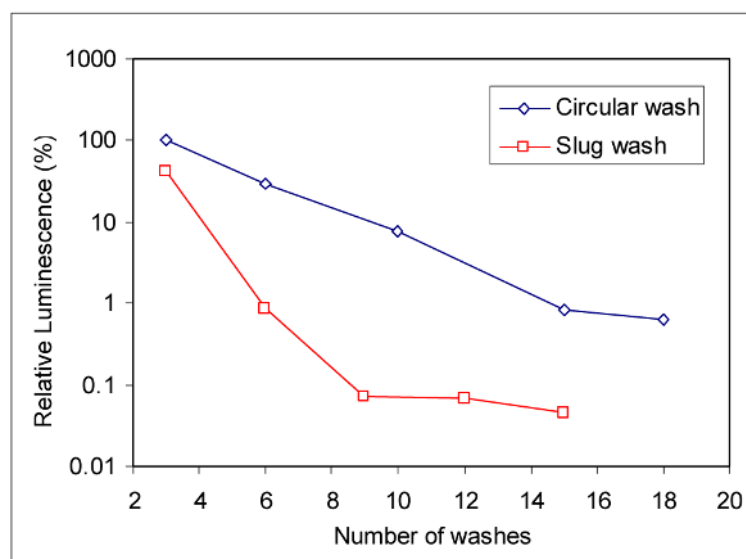


Figure 8. Comparison of washing protocols between slug shaped and circular shaped wash droplets

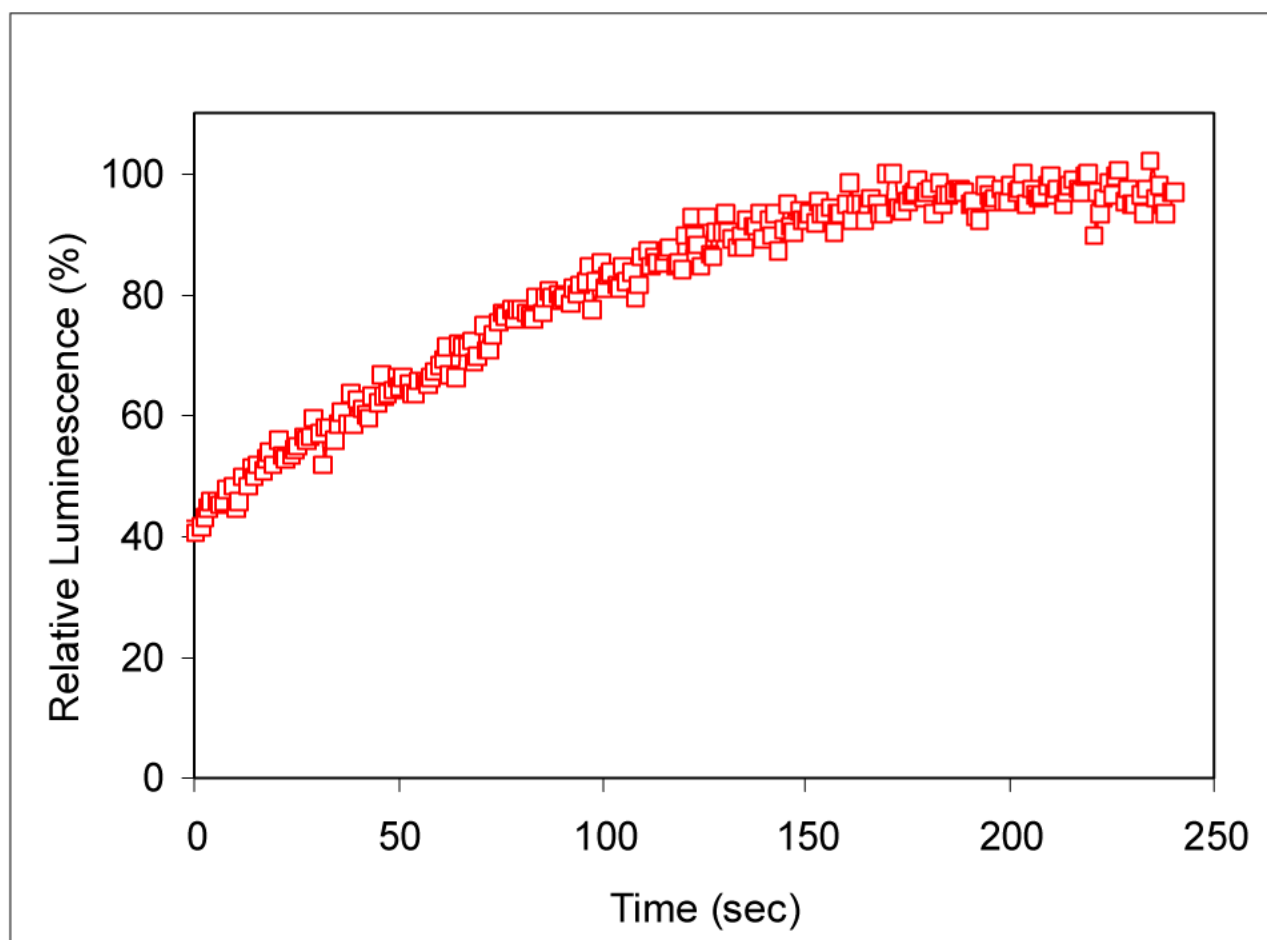
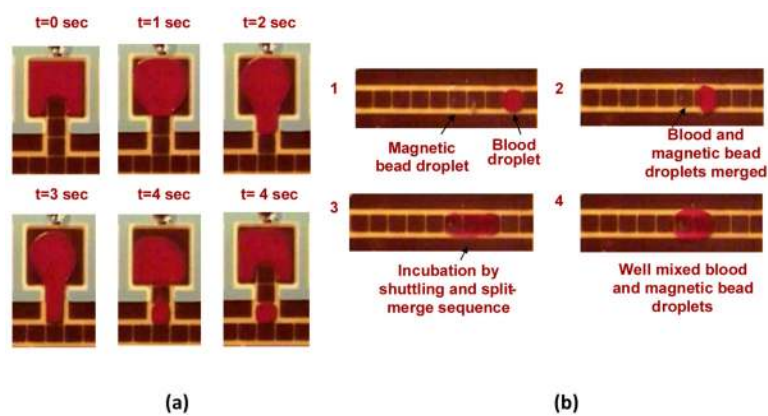


Figure 9. Chemiluminescence kinetics for TnI (100 ng/mL)



**Figure 10. (a) Dispensing of whole blood from a reservoir on a digital microfluidic chip, (b) Incubation of whole blood droplet with a magnetic bead droplet containing antibodies**

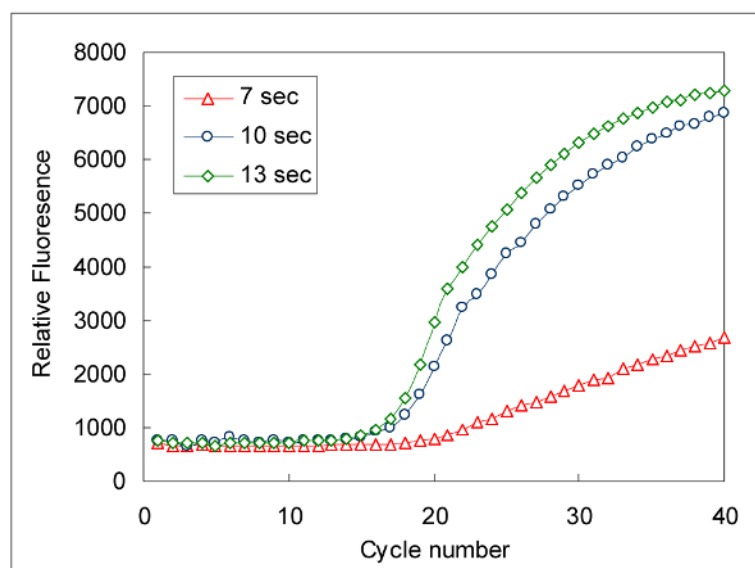
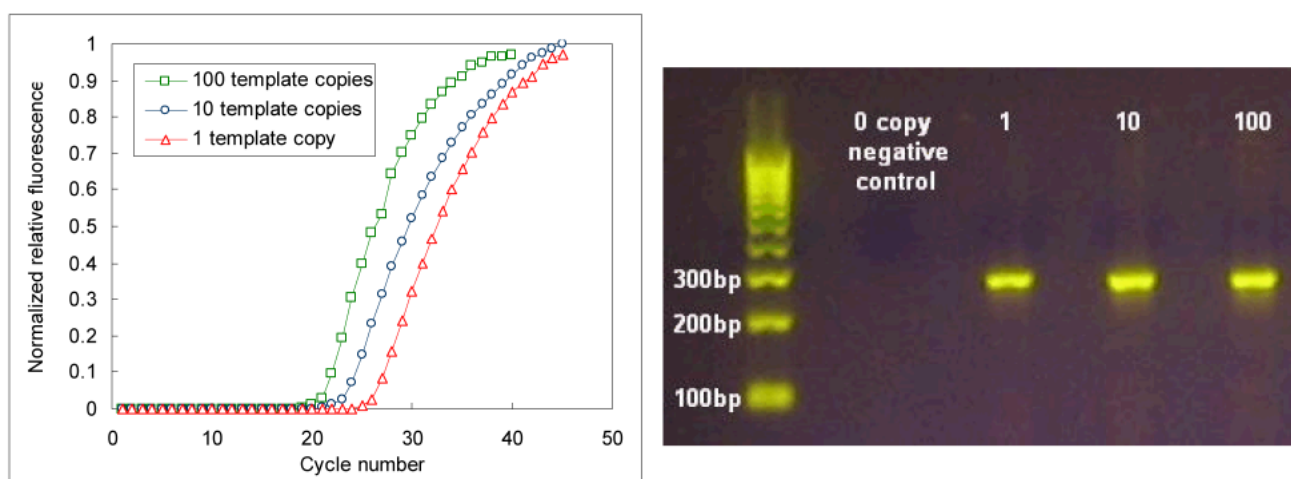
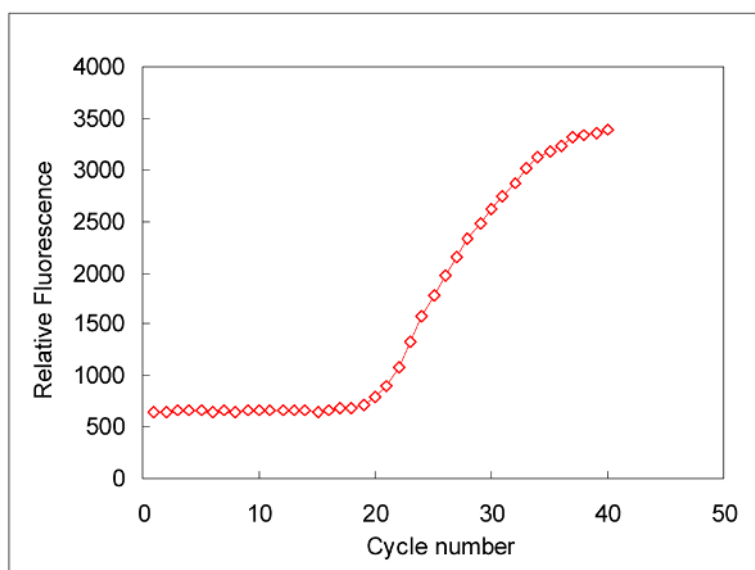


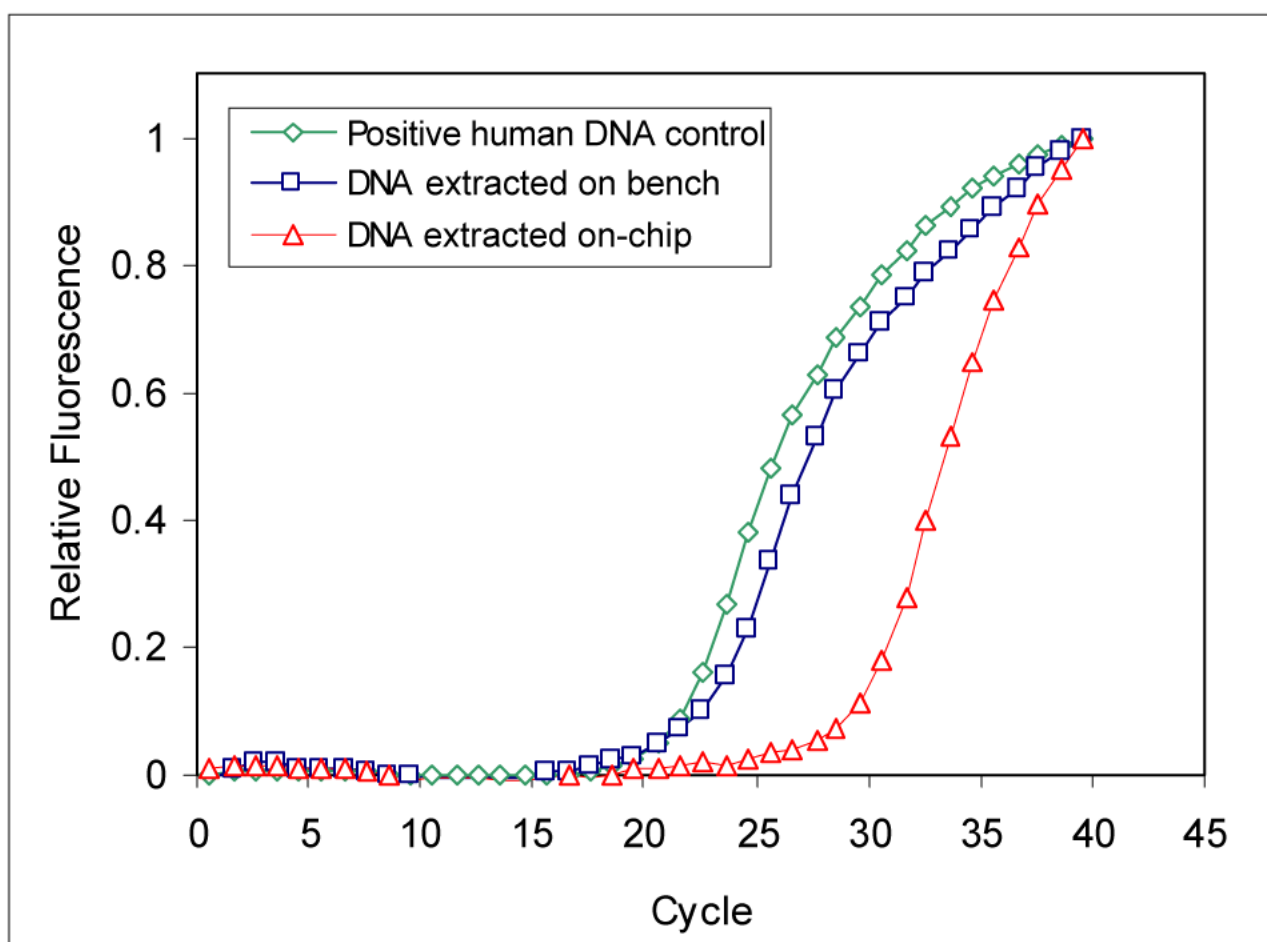
Figure 11. MRSA real-time on-chip PCR with different combined annealing/denaturation times



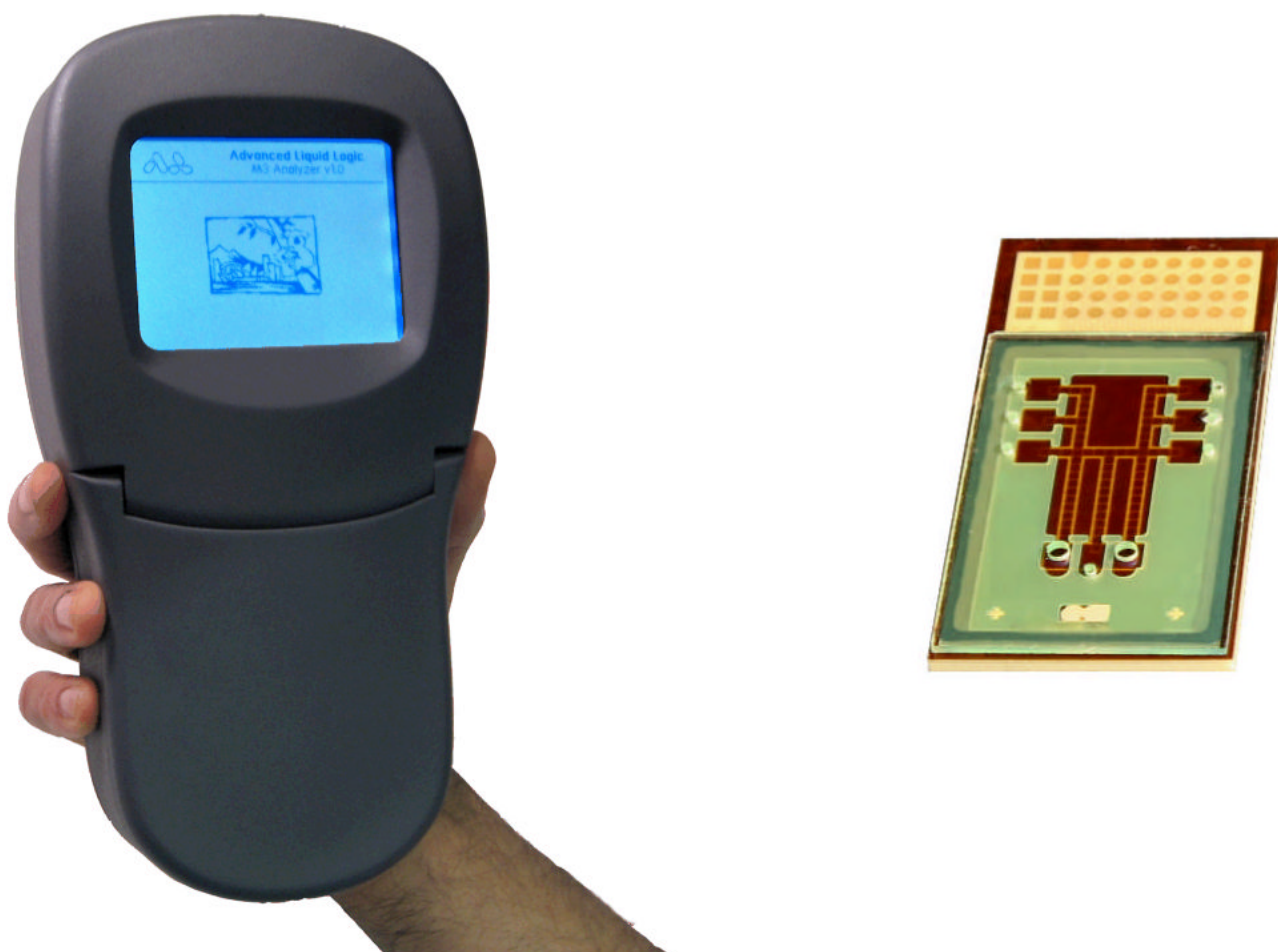
**Figure 12. Real-time on-chip PCR titration of samples (left) and corresponding gel images (right) with different number of *Candida* genome copies**



**Figure 13. Real-time PCR results for the MRSA cell samples prepared both off and on-chip and entirely analyzed on-chip**



**Figure 14.** A quantitative PCR plot of the DNA extracted from whole human blood on chip (triangles, with  $C_t = 28.8$ ) compared against that of DNA from an on-bench extraction (squares, with  $C_t = 23.3$ ) and a positive human DNA standard (circles, with  $C_t = 21$ ). In all the cases, PCR is performed on an iQ5 multicolor real-time PCR detection system



**Figure 15.** Picture of hand held instrument along with its cartridge. The cartridge size is 3.04 cm× 6.09 cm

**Table 1**  
**Specifications of the cartridges used for different applications**

Parameter	Rapid Immunoassays	Rapid DNA amplification	DNA sample preparation
Electrode pitch	1.125mm	1.125mm	1.125mm
Droplet height	185µm	185µm	185µm
Unit droplet volume	~300nL	~300nL	~300nL
On-chip reservoirs	12 samples, 8 reagents	12 samples, 8 reagents	9 reservoirs
Off-chip reservoirs	2 wash & 1 substrate	2 wash	None
Overall chip size	8.55 cm × 12.78 cm	8.55 cm × 12.78 cm	3.04 cm× 6.09 cm
Electrical inputs	64	64	32
Filler fluid	Silicone oil	Hexadecane	Silicone oil