ABSTRACT
A digital microfluidic multiwell platform for performing 96 heterogeneous immunoassays based on handling of magnetic beads is presented in this paper. The digital microfluidic multiwell plate can be configured in the software to perform a single immunoassay on several samples or several immunoassays on a single sample or combination thereof. We have demonstrated a one-plex immunoassay on 12 samples and a 4-plex immunoassay on a single sample.

KEYWORDS: Immunoassay, Digital Microfluidics, Multiplexing

INTRODUCTION
Immunoassays are among the most sensitive and specific analytical methods that are routinely used in clinical laboratories and other research applications. Currently these are performed using macrofluidic equipment utilizing large liquid volumes with mechanically complex robots at high cost and comparatively low speed. Magnetic beads have been successfully utilized in several commercial immunoassay analyzers but are not as prevalent in microfluidics systems. Wang et al [1] demonstrated separation of magnetic particles in a droplet. Recently, our group demonstrated the feasibility of performing a magnetic bead immunoassay on a digital microfluidic system [2]. Here, we demonstrate the capability of our digital microfluidic system to perform up to 96 immunoassays by spatial multiplexing where several immunoassays are performed simultaneously in independent droplet reactors. Cross reactivity between antibody pairs is minimized in such a spatial multiplexing scheme.

EXPERIMENTAL
Sandwich immunoassays were performed on the digital microfluidic cartridge, where a sample droplet (antigen) was dispensed and mixed with two reagent droplets (one droplet with capture antibodies on magnetic beads and another droplet with ALP-labeled reporter antibodies). After incubation, the magnetic beads were washed by merging and splitting wash droplets. Finally the washed droplet with magnetic beads is mixed with a substrate droplet that produces chemiluminescence.

All the immunoassays were performed on a digital microfluidic multiwell plate (Figure 1) that has 8 reagent reservoirs and 12 sample reservoirs. The sample reservoirs on this digital microfluidic cartridge were placed in the same pitch as that of an SBS 384-well plate (4.5mm) and the reagent reservoirs to match that of an SBS 96-well plate (9mm) so that the cartridge is compatible with high throughput robotic systems. Each reagent and sample reservoir is designed to dispense at least 12 and 4 droplets respectively. Although a single reagent reservoir can contain both the capture and reporter antibodies, two reagent reservoirs were used to hold the magnetic
beads with primary capture antibodies and the secondary reporter antibodies for a run of 12 assays. The well for wash buffer (Figure 1b) attached to the chip was tested to dispense >1500 droplets without requiring reloading, which is sufficient for performing all the immunoassays. This digital microfluidic multiwell plate is software programmable where the droplets can take any path and all the droplet operations including incubation and washing can be performed at any location. However, each assay is performed on a separate pathway of electrodes in order to perform spatial multiplexing and increase parallelization of operations. All the steps involved in an immunoassay, including sample and reagent aliquoting, incubation with antibodies, bead washing and enzymatic detection, were completely automated on the chip.

![Figure 1(a) Schematic of the multiwell chip with 8 reagents wells and 12 sample wells (b) A multi-well plate format digital microfluidic cartridge for performing 8-plex immunoassays on 12 samples for a total of 96 immunoassays. The chip is replete with wash reservoirs, waste reservoirs, and substrate reservoirs for ELISA.](image)

RESULTS AND DISCUSSION

Eight six-point standard curves (0-100 ng/mL) were obtained (Figure 2a) in four runs for a cardiac marker, Troponin I, where 12 immunoassays were performed in each run on the same cartridge. This demonstrates the feasibility of performing single plex assays on several samples. The data obtained from the eight standard curves for TnI were fit using a 4-parameter logistic function in SigmaPlot (Figure 2a). No weighting parameters were included in the fit and the error bars indicate standard error of the mean for each standard measured in different runs.

To evaluate the intra-cartridge variability, 12 assays were performed on the same cartridge on another cardiac marker, CK-MB, at 30 ng/mL where each assay was performed on samples dispensed from each of the 12 sample reservoirs on the cartridge. The intra-cartridge CV for the data was <6% (Figure 2b). The imprecision is an aggregate of several sources of variabilities including droplet volumes between reservoirs and beads dispensed per droplet.

Additionally, several multiplexed assays were performed from a single plasma sample spiked with known concentrations of Troponin I (TnI), Myoglobin, CK-MB. In addition to core lab clinical diagnostic applications, where single plex assays are generally performed on several patient samples, the digital microfluidic multiwell
CONCLUSIONS

The versatility and programmability of the digital microfluidic multiwell plate lends itself readily adaptable to high throughput research environments as well as single-use point-of-care applications. This is the first demonstration of 1000’s of droplet operations on a digital microfluidic chip and a high order of spatial multiplexing of immunoassays in droplets. Work is ongoing to improve the time to results, increase parallelization, improve droplet traffic management, and to integrate PCR (“Rapid detection of Methicillin-resistant staphylococcus aureus (MRSA) using Digital microfluidics”, Presented at MicroTAS 2008 by Hua et al.) and enzymatic assays.

Figure 2(a) Standard curve (average from n=8) for TnI generated on the same chip. Error bars indicate standard error for 8 runs (b)Twelve immunoassays performed on the same cartridge for CKMB (CV=6%)

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