Miniaturized ELISAs with a Novel Flowchip Device

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INTRODUCTION

Enzyme-linked immunosorbent assays (ELISA) are one of the most common assay types used in drug discovery and development. Immunoassays are also used extensively for study of chronic and infectious diseases, oncology, biomarkers, and other areas. Limitations of this technology are well known. The assay times can be 4-6 hours, sometimes including overnight incubations. The protocols are complicated and include many wash steps that make automation challenging. Finally, standard 96-well formats need relatively large amounts of expensive antibodies and other reagents. Use of microfluidics can overcome these limitations. The large surface-to-volume ratio of microfluidic channels significantly reduces reagent diffusion times and, correspondingly, incubation times. The small channel volume means that assays can be performed with low-microliters of sample. Hydrodynamic effects in the channels increase reagent removal efficiency and eliminate need for multiple wash steps. Here we present a novel flowchip device using microfluidic channels and a proprietary valve-less fluidic switching technology that has been optimized for the ELISA format. Results of two assays will be presented: a competitive assay for the environmental biotoxin microcystin and a sandwich assay for total histone H3. Performance comparisons for assay time, reagent use, and sensitivity will be made to assays run in a 96-well plate format. This new format offers potential for fully automated immunoassays that can be run in under 1 hour using a fraction of the reagent volumes currently required. It also fits seamlessly into a typical ELISA workflow with easy conversion of protocols that have been optimized for 96-well or 384-well plates to the flowchip format.

BACKGROUND

The utility of microfluidics has been demonstrated for a variety of assay types. Key advantages include:

- Low sample and reagent usage
- Faster assay results
- Ability to create unique cellular structures

Here we show performance from a microfluidic flowchip optimized for the ELISAs. Flowchips have been fabricated in both PDMS (Fig. 1) and thermoplastics using a proprietary valve-less fluidic switching technology.

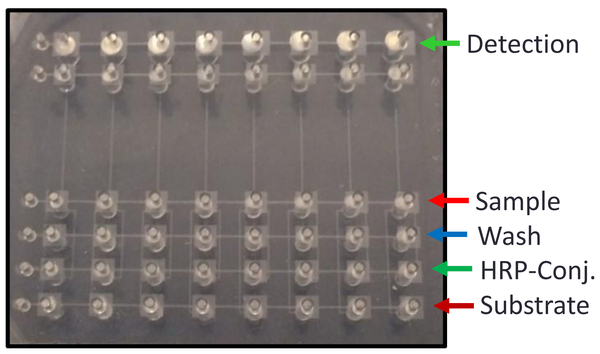

Figure 1. Flowchip configuration for absorbance based competitive immunoassay. Image shown is of a device fabricated with PDMS.

AUTOMATION

A key challenge for microfluidics has been automation of the devices. PDMS is a versatile material, but flowchips made from PDMS typically require manual assembly and are not cost effective. Thermoplastics are much more well-suited for automation and can be manufactured in high-volume. We have integrated our novel flowchip with an automated assay processing system. Key aspects of the system include:

- Valve-less fluidic switching
- Thermoplastic based flowchip
- Automated system for assay control
- Uses 96- or 384-well format spacings
- Tablet/laptop driven with intuitive user interface

MICROCYSTIN ASSAY

Microcystin is a known environmental biotoxin that is found as a contaminate in water supplies. Detection of microcystin at the sub-part per billion (ppb) range is required for water safety testing (regulatory limit = 1 ppb). We developed a competitive assay for detection of microcystin using ELISA method with absorbance readout (Fig 2). Comparison was made between assay performance in a flowchip device and a commercially available kit (Quantiplate Kit for Microcystins, Envirologix, Portland, ME). The protocol was optimized for the flowchip device using a microcystin Adda specific capture antibody (ENZO Life Sciences, Farmingdale, NY) and HRP conjugated microcystin LR.

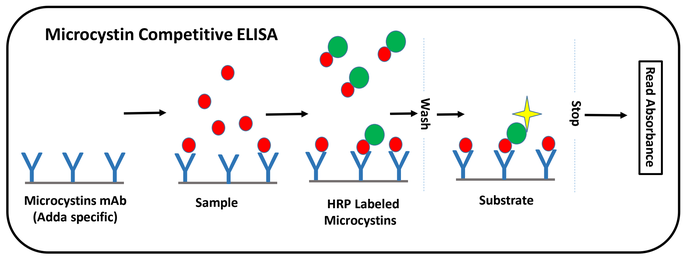

Figure 2. Cartoon showing workflow for competitive microcystin assay.

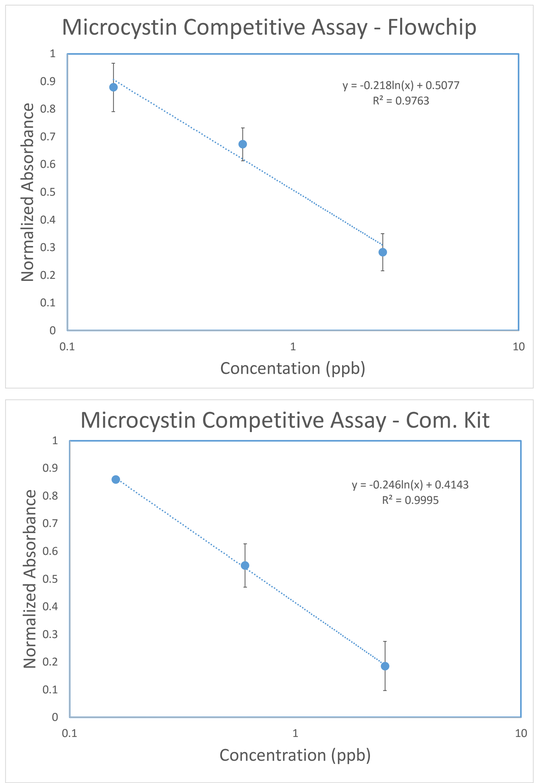

Figure 3. Immunoassay results for detection of microcystin LR. Top: Results with PDMS flowchip device. Bottom: Results for a commercial kit run in 96 well plate format. Absorbance measurements were done using a custom 450nm optical absorption meter with a 6mm path length

	96 Well Plate	Flowchip
Capture Ab Loading Time	Overnight	1 Hour
Assay Time (Sandwich)	4-6 Hours	<1 Hour
"Hands-on" Time	>1 hour	<10 min
Sample Volume	100-200 µL	10-20 µL
Read Mode	Absorbance	Absorbance
Limit of Detection	5-15 ng/well	5-15 ng/well

Table 1. Comparison of assay performance parameters between 96-well plate and flowchip formats.

HISTONE H3 ASSAY

Histone proteins are crucial to the organization of genomic DNA and post-translational modifications of histones are important regulators of genome function. Histone H3 is a core component of the nucleosome and determination of its levels is important for epigenetics studies. We optimized the performance of a commercially available Total Histone H3 assay kit (Active Motif, Carlsbad CA) on a flowchip device and compared performance to the same assay run in a 96-well plate. The capture antibody used for the flowchip (Histone H3 mAb, Active Motif) was the same as used in the Total Histone H3 Kit. Detection was performed with a biotin-histone H3 antibody and streptavidin-HRP.

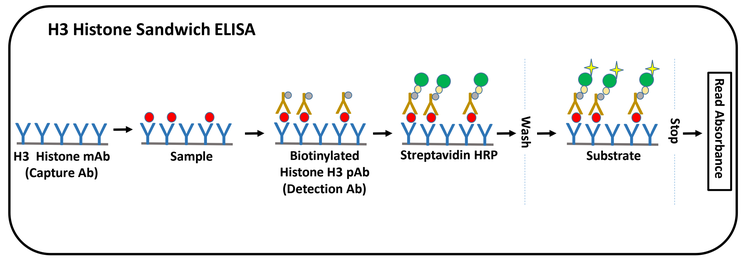

Figure 4. Cartoon showing flowchip workflow for total histone H3 sandwich assay. 96-well plate protocol has additional wash steps.

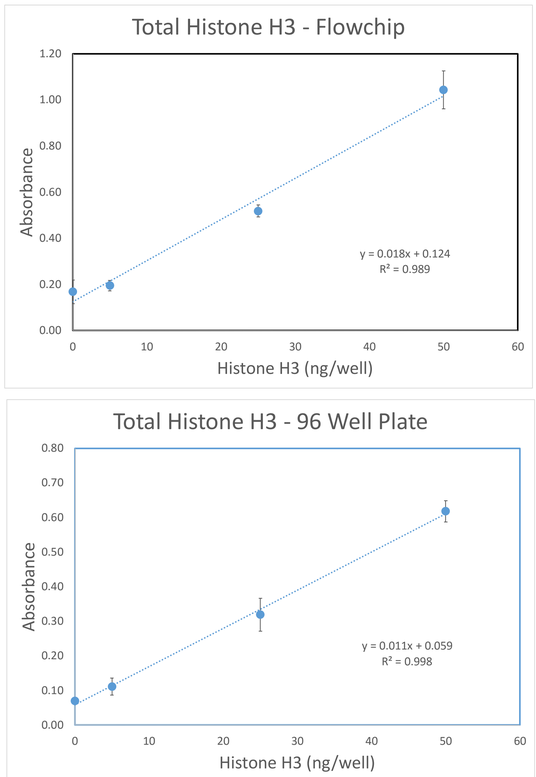

Figure 5. Immunoassay results for total histone H3. Top: Results from running assay through thermoplastic flowchip device. Bottom: Results from running assay in 96 well plate. Absorbance measurements were done using a custom 450nm optical absorption meter with a 6mm path length.

CONCLUSIONS

- We have developed a novel flowchip system using valve-less fluidic switching for automated immunoassays
- The flowchip system provides similar sensitivity to a multiwell plate format while significantly reducing reagent use and time-to-results
- Assays can be converted from 96-well format to the flowchip in a straightforward manner minimizing assay development time

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