Low-volume Immunoassays Using Novel Flowchip System

Byungrae Cho[1] Neil Bristol[1]; Oksana Sirenko[2]; and Evan F Cromwell[1]
[1]Protein Fluidics, Inc. 875 Cowan Rd, Suite B, Burlingame CA 94010, [2]Molecular Devices, LLC, 3860 N First Street San Jose, CA 95134

INTRODUCTION

Immunoassays are one of the most common assay types used in clinical diagnostics, drug discovery, in vivo pharmacology and other biochemical studies. Microfluidic-based systems have been successfully commercialized that reduce reagent usage and lower assay times. The large surface-to-volume ratio of microfluidic channels reduce reagent diffusion times and, correspondingly, incubation times. The small channel volume means that assays can be performed with low-microliters of sample. However, currently available systems typically require expensive instrumentation and suffer from high consumable cost. We report here on a novel flowchip system that uses Valve-less Fluidic Switching (VLFS) to move reagents through microfluidic channels without the use of integrated multi-layer valves and pumps. The system uses a sealed single-layer device with hydrophobic capillary burst valves to control fluid movement. The flowchip is placed in a benchtop system that applies pressure differentials across reagent wells to precisely move reagents through the channel network and automatically perform all assays steps. An injection molded cyclic-olefin (COC) flowchip designed for enzyme-linked immunosorbent assays (ELISA) has been developed and demonstrated for several common diagnostic tests. Results for IL-6, TNFα, and IFNγ assays will be presented. Performance of the system is compared to assays run on 96-well plates using the same antibodies and reagents. Complete assay times (Capture to Substrate) are reduced from a 2-day workflow to under 3 hours. Reagent and antibody usages are reduced by 5 times or more. This new format offers potential for fully automated immunoassays that can be run using a fraction of reagent amounts in total assay times suitable for assay development, antibody pair screening, or in vivo pharmacology.

PUMA SYSTEM WORKFLOW

The Pu·MA System is a practical and affordable benchtop instrument that runs ELISA assays using your own antibodies, or precoated flowchips in a streamlined workflow. The Pu·MA System has been designed to fit seamlessly into your current laboratory workflow.

- Runs complete ELISA in < 3 hours with "hands-off" processing
- Reduces sample and reagent volumes to 10 - 20 µl
- Works with your existing ELISA kits and antibody pairs

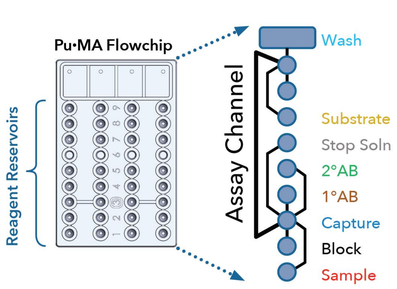
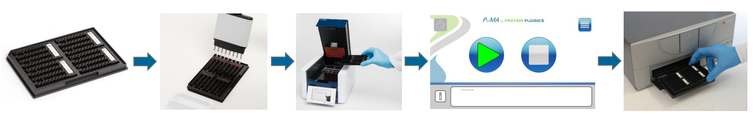


Figure 1. Flowchip setup for a Sandwich Immunoassay. All reagents are pre-loaded then the Pu·MA System automatically performs all assay steps including wash and substrate incubation.

Pu·MA System Workflow – 2-3 hours "hands-off"



Standard ELISA Workflow – 2 Days

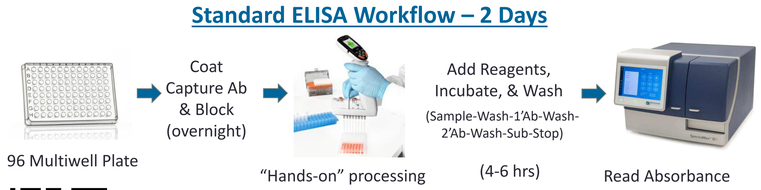


Figure 2. ELISA workflow for automated Pu·MA System and general protocol for complete 96-well plate ELISA.

MICROFLUIDIC IMMUNOASSAY

- High Signal – ***Large surface area for antibody binding*** (~30mm^2)
- Small channels – ***Low sample and reagent usage*** *(<20µl/well)*
- Large surface-to-volume ratio – ***Enhanced kinetics, Faster assay results***
- Hydrodynamic effect & efficient fluid removal – ***Less Wash Required***

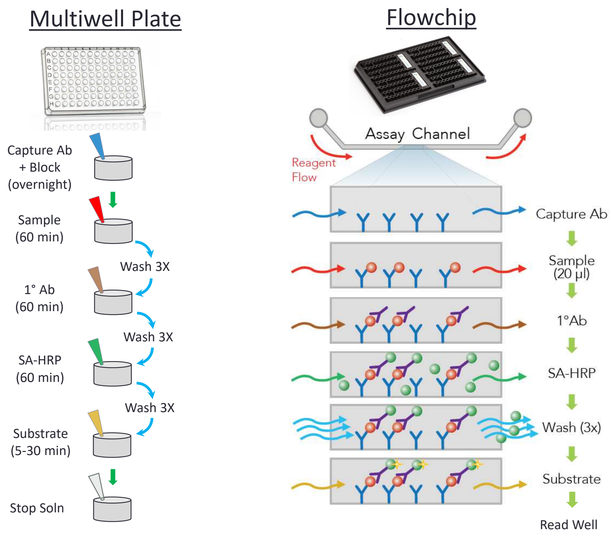


Figure 3. Cartoon of automated assay steps in an ELISA protocol. In the Multiwell Plate protocol (Left) reagents are added sequentially with multiple wash steps. In the Flowchip protocol (Right) reagents are pre-loaded into reservoirs then automatically sent through the Assay Channel to perform the assay steps. Substrate is incubated in the Assay Channel then sent into the Read Well.

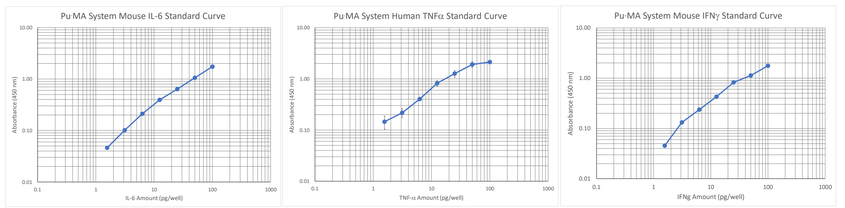
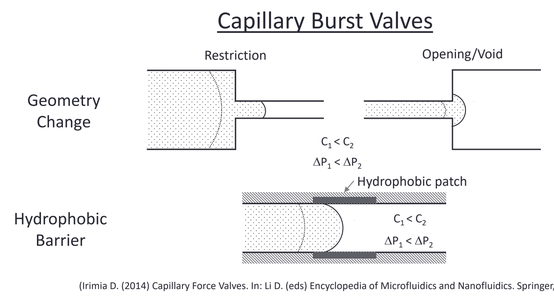


Figure 4. Standard concentration response curves for three common cytokines. Assays were run using antibodies and avidin-HRP from BioLegend ELISA Max Kits. Assay buffers and other reagents were optimized by Protein Fluidics for use in PuMA System.

VALVE-LESS FLUIDIC SWITCHING

The PuMA System uses ***Valve-Less Fluidic Switching*** (VLFS) a patent pending technology that allows precise fluid control in a microfluidic device without the need of mechanical microvalves or integrated moving parts. Benefits include:

- Enables a broad range of immunoassays and other life science applications
- Programmable & automated flow switching and combinatorial mixing
- Compatible with injection molded thermoplastics for large scale production
- Uses hydrophobic barriers to control fluids – no integrated valves or pumps



(Irimia D. (2014) Capillary Force Valves. In: Li D. (eds) Encyclopedia of Microfluidics and Nanofluidics. Springer, Boston, MA)

Figure 5. Methods for controlling fluid movement in microfluidic device using VLFS

THP-1 INFLAMMATION ASSAY

Differentiation of THP-1 cells into macrophages was quantified by measurement of number of adherent cells using an ImageXpress® Pico Automated Cell Imaging System. The amount of IL-8, IL-1β and TNF-α in cell supernatants was quantified using a low volume, microfluidic-based Pu·MA System ELISA. The PuMA System runs ELISAs using small sample volumes (10-20 µl with existing antibody pairs. This enhances the ability to measure multiple cytokines where supernatant volume is limited.

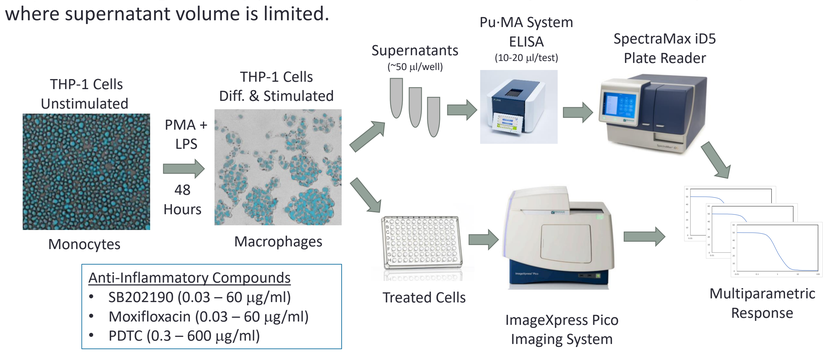


Figure 6. Cartoon of Multiparametric Inflammation assay workflow.

- THP-1 cells were plated 20,000 cells per 96well and incubated for 48 hr. Next they were stimulated with a mix of PMA & LPS for 24 hr (0-5 pg/mL of PMA, and 0-100 pg/mL LPS; all from Sigma).
- Anti-inflammatory compounds were added 2 hr prior to cytokine stimulation
- After incubation, 60 µl of supernatant was taken for ELISA analysis from each well. The samples were analyzed fresh or stored at -70 C for subsequent analysis.
- Supernatants were diluted 3:1 in assay buffer and analyzed for IL-8, TNFα, and IL-1β using the Pu·MA System flowchips and reagents (all Ab pairs from BioLegend).

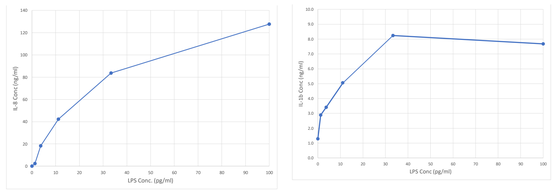
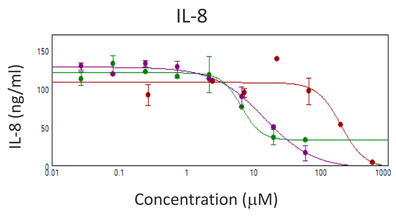


Figure 7. Increase in cytokine secretion as a function of LPS concentration. Amount of IL-8, IL-1β, and TNFα at lowest (Unstim) and highest LPS concentrations (Stim) are provided in tables.

IL-8 Pos/Neg (ng/ml)		
	Avg	SD
Stim	127	0.2
Unstim	4.2	2
Fold Ind	30	

IL-1b Pos/Neg (ng/ml)		
	Avg	SD
Stim	7	0.5
Unstim	1.2	0.2
Fold Ind	6	

TNFa Pos/Neg (ng/ml)		
Stim	3	0.1
Unstim	0.2	0.1
Fold Ind	15	



EC_{50}, µM	IL-8	IL-1β	TNFα
PDTC	201	42	63
SB202190	6.6	9.8	6.4
Moxifloxacin	17.4	9.9	17.9

Figure 8. Concentration dependent effects on IL-8 and number of adherent cells by three anti-inflammatory compounds. EC_{50} values derived from a 4-P fit are given in the table above.

CONCLUSIONS

- We have demonstrated a novel microfluidic-based automated low-volume ELISA system that provides results comparable to 96-well platforms.
- The Pu·MA System performs immunoassays with existing ELISA antibody pairs using microfluidic flowchips that reduce reagent use and improve time-to-results
- Utility of the system for analyzing cytokines in supernatants was demonstrated using a THP-1 cell inflammation assay.
- The PuMA system is ideal for applications where sample volumes are limited such as supernatants from precious cells or blood draws from small animals.

PROTEIN FLUIDICS