From Academic Research to Commercialization:

ULTRA-HIGH SENSITIVE IMMUNOASSAYS USING MICROFLUIDIC PLATFORMS FOR IN VITRO DIAGNOSTICS (IVD) POCT

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ABSTRACT
A simple spiral microchannel-based reaction cell, which delivers the well-established benefits of microfluidics in terms of high sensitivity, rapid assays and ultra-low sample volumes, is introduced and discussed for its commercial application in this talk. The technology has been exhaustively validated and is well into commercialization to an ultra-high sensitive immunoassay platform, which is a simple yet elegant integration of microfluidics into immunoassay systems. In this talk, the microfluidic technology fundamentally developed from academic research, which can be evolved toward its commercialization, is presented and discussed.

KEYWORDS: Microfluidic platform for immunoassay; ELISA; in vitro diagnostics (IVD), POCT

INTRODUCTION
Microsystem and BioMEMS Laboratory at the University of Cincinnati has developed numerous microfluidic devices and technologies which include micro reactors and incubators [1], microdispensers and mixers [2-4], microvalves and pumps [5], injection molded polymer lab-on-a-chips [6, 7], and blood/plasma separators [8, 9]. The developed microfluidic devices and platforms have been applied for the development of magnetic bead-based sampling and electrochemical detection [10], magnetic bead-based lab-on-a-chip for immunoassay [10, 11], polymer smart lab-on-a-chip for blood analysis [6, 12], microchannel-based 96-well microplate [13-15], and POCT [16]. Figure 1 shows select examples of microfluidic devices and polymer lab-on-a-chips developed at the University of Cincinnati.

![Figure 1. Microfluidic devices and lab-on-a-chips developed at University of Cincinnati: (a) magnetic separator and detector system on fluidic mother board [1]; (b) polymer smart lab-on-a-chip for blood analysis [6] and (c) polymer lab-on-a-chip for Hematocrit [8].](image)

The benefits of microfluidics, in terms of small volumes and high surface to volume ratio (for rapid reactions); have been well understood for over a decade. The key distinguishing and unique attribute of the Optimiser™ reaction cell (developed at Siloam) [13-15] is the standardized “world-to-device” fluidic interface compatible with existing liquid handling systems. This offers a practical solution for delivering the benefits of microfluidics without large scale capital investment. The incredibly versatile Optimiser™ platform offers; for the first time ever, the seamless capability for a single platform technology for the complete spectrum of immunoassay testing for research, lab-based in-vitro diagnostics (IVD), and POCT applications.
OPTIMISER™ TECHNOLOGY PLATFORM [13-15]

Optimiser™ as described in Figure 2 uses a microfluidic immunoreaction chamber thereby delivering the well-established benefits of microfluidics in terms of high sensitivity, rapid assays and ultra-low sample volumes. Reagents added to well are transported via capillary action to absorbent pad under plate. Liquid is trapped in the microchannel by capillary forces. Adding next liquid breaks capillary barrier and first liquid is “flushed” out to pad. All assay reactions occur within microfluidic reaction chamber. Microfluidic reaction chamber with 200 um x 200 um cross-section allows for extremely rapid diffusion. Approximately 75 % of maximum binding occurs within first 10 seconds leading to ultra-fast assays with total assay time of 10 min. Binding (functional) surface area of microfluidic reaction chamber is similar to conventional ELISA platforms but with much smaller volume; the (surface area:volume) ratio is 50-fold higher. Coupled with rapid incubation, this allows for up to 1000-fold sensitivity gain as shown in Figure 2 (c).

![Figure 2](image)

Figure 2. Optimiser™ microplate illustration: (a) Optimiser™ microplate with magnified view of one cell; (b) manufactured Optimiser™ microplate; and (c) immunoassay performances for human IL-4 [13-15].

POINT-OF-CARE TEST (POCT) SYSTEM [13-16]

Siloam’s has applied Optimiser™ technology to the first-ever open source POCT system (TROVA™) as shown in Figure 3 [16]. The key component of this system is the microfluidic test cartridge incorporating 5 Optimiser™ reaction cells and 3 storage reservoirs. This design allows the test cartridge to be configured for multiple types of tests: for instance up to 4 analytes and control or for 2 analytes across a 5-log dynamic range. The POCT cartridge and Optimiser™ microplate use identical Optimiser™ reaction cells and assays can be developed on microplate format and rapidly and seamlessly transferred for POCT use.

![Figure 3](image)

Figure 3. TROVA™ POCT analyzer: (a) schematic view of one cell; (b) cartridge and POCT analyzer; and (c) performed beta-HCG immunoassay [16].

The TROVA™ POCT analyzer is a modular system with precision pump, chemiluminescence detection module, linear motion stages, and touch screen user interface. TROVA™ POCT system is designed for use in Emergency room labs and Physician’s office labs and is intended for use with minimal training (CLIA waived use). The user simply adds ~ 20-25 µl of sample (whole blood, serum/plasma etc.); initiates the test by pressing the START button and the system handles the entire test procedure and reports the result. The assay performance for beta-HCG is shown in Figure 3(c).
CONCLUSION
The distinctive nature of simple spiral microchannel-based reaction cell performs rapid and high sensitive immunoassays, allowing significant reagent and time savings. With this high sensitivity, the combination of lab-on-a-chips and microfluidics is very suitable for the point-of-care testing (POCT) diagnostics with needs for a rapid turn-around-time, high sensitivity and low assay cost. In conclusion, the simple spiral microchannel-based reaction cell, as a new game changer, opens a new realm of immunoassays and ELISA for IVD clinical diagnostics and POCT applications.

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