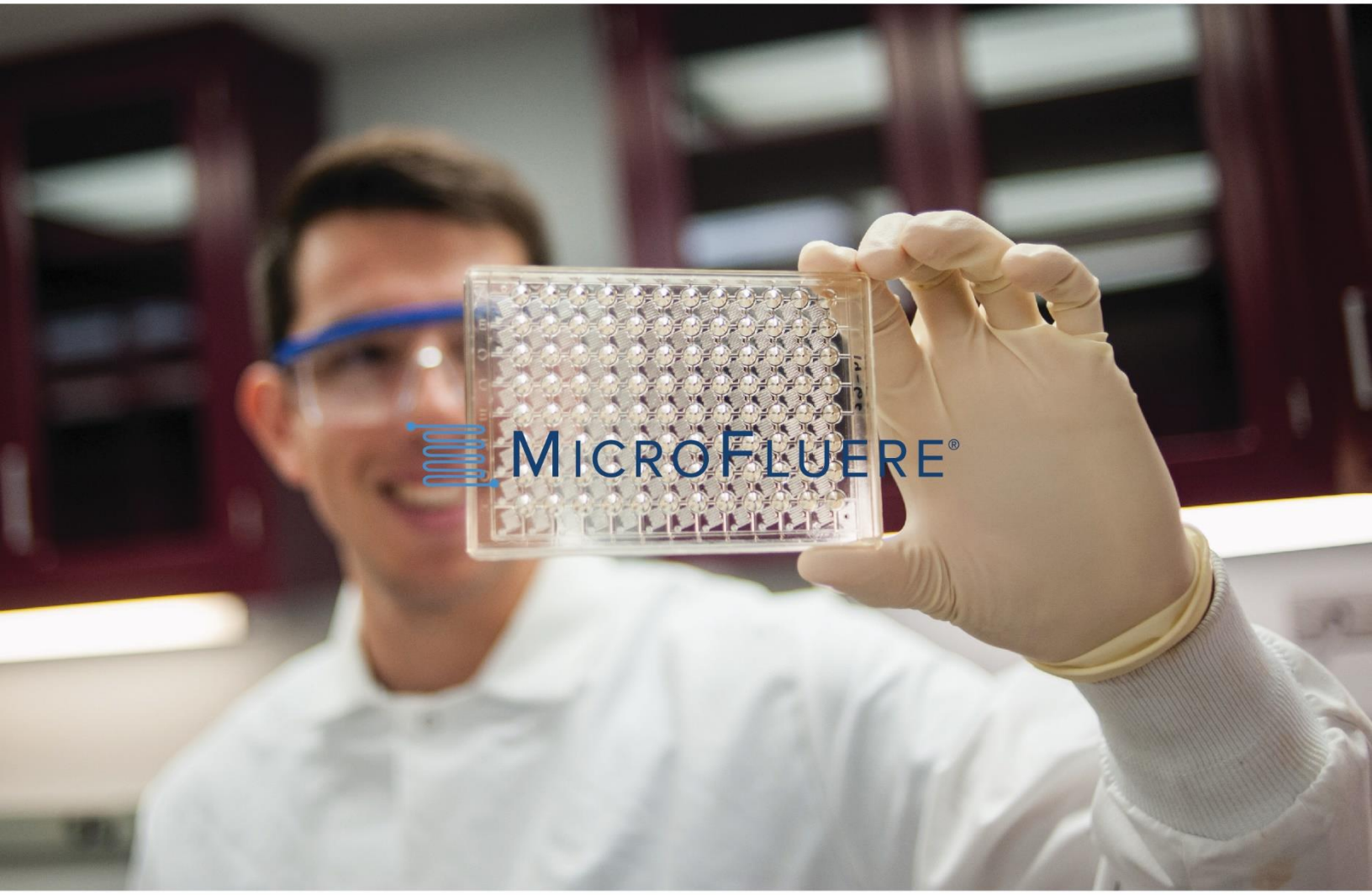
OPTOFLUIDIC BIOASSAY



674 S. Wagner Road
Ann Arbor, MI 48103
https://www.optobio.com/
(734) 252-9645

Optofluidic Bioassay, LLC has developed two cutting edge products; (1) MicroFluere® and (2) Xpress ELISA. Both products bring significant improvement to the conventional ELISA (enzyme-linked immunosorbent assay) test workflow.

(1) MicroFluere®

MicroFluere®, novel microfluidic 96-well ELISA plate, has validated with a few biomarkers and has demonstrated superior results in sample/reagent size, workflow and assay time in ELISA tests in comparison with a standard 96-well plate. The benefit of using Optofluidic Bioassay's microfluidic plate includes 85% time savings per test by eliminating steps in the ELISA workflow at shorter reaction times and up to 80% cost savings by using only 20% of the sample/reagent size—all without requiring any modification to their current workflows or equipment. Please see detail in the section of MicroFluere®. The product is currently available to sell. Please contact us if you like to try free samples.

(2) Xpress ELISA

Another product is low cost Xpress ELISA system that includes standalone desktop automated ELISA machine and disposable components. It will suit research labs who has limited space, cannot afford a fully automated ELISA (expensive and bulky) system and incur high costs to outsource their tests. Being automated liquid handling module in the system can eliminate human pipetting error. Therefore, it can rapidly produce reliable and repeatable with high precision and accuracy results (see detail in the section of Xpress ELISA). Moreover, it needs only a small fraction of sample that used in traditional ELISA. We anticipate that the product will be available in market by 2020.

MicroFluere®

for

Faster, Less Expensive, and More Efficient ELISA

MicroFluere® plate uses 20% of sample and reagents of that used for a traditional plate and achieves a larger dynamic range in 4-6X shorter time.

Background

Since the early 1970's, Enzyme Linked Immunosorbent Assay (ELISA) has become a powerful and standard method for detecting and quantifying specific analytes in complex liquid mixtures. It is widely used in clinics and industrial/research labs. However, ELISA using a traditional microplate (the most common format of the traditional microplate used in ELISA is a 96-well plate having 12 columns x 8 rows) suffers from a few major drawbacks: (1) long testing time (3-6 hours), which makes the ELISA almost useless when dealing with situations such as emergency care and continuous monitoring (*i.e.*, sepsis) where the results should be obtained within 15-30 minutes; (2) large sample and reagent consumption (50-100 μL per well), which poses a significant challenge when samples are limited and adds high costs to customers; and (3) low dynamic range, which makes it difficult to quantify a wide range of concentrations without sample dilution.

To address the aforementioned shortcomings, Optofluidic Bioassay, LLC has developed a novel microfluidic well plate, called MicroFluere®, which greatly improves ELISA performance. The plate is made of plastic (i.e., clear or black polystyrene) and consists of 96 flow-through microfluidic units arranged in the standard ELISA 96-well format so that MicroFluere® is fully compatible with existing plate readers. The plate significantly improves the conventional ELISA test workflow by eliminating many of the time consuming steps and reducing incubation times. More importantly, it enables the researcher to use only 20% of the original sample/reagents

compared to that used for a traditional 96-well plate and achieve a larger dynamic range with the same lower detection limit. Additionally, MicroFluere® allows the assay to be completed in 4-6X shorter time.

Technology

A MicroFluere® plate comprises 96 microfluidic units arranged in the same format as in a traditional 96-well plate. The plate's footprint, height, and bottom and outside flange are in compliance with the ANSI (American National Standards Institute) and SLAS (Society for Laboratory Automation and Screening) 96-well plate standard. Therefore, it can be read with existing plate readers without any modifications to the readers.

As shown in **Figures 1** and **2**, each microfluidic unit has a liquid inlet, an optically clear detection channel containing micro-posts, and a liquid outlet. In the microfluidic unit, samples and reagents flow from a funnel-shaped structure through a micro-post array embedded microfluidic channel.

The liquid can be withdrawn from an opening outlet using a wicking method or pressure differential. The optical signal is acquired at the center of the microfluidic loop located at the standard plate reader optical excitation/collection position. Since the inlet and outlet are offset from the detection area, there is no interference to the optical signal caused by sample/reagent residuals at the inlet (*i.e.*, funnel-shaped structure) or the outlet.

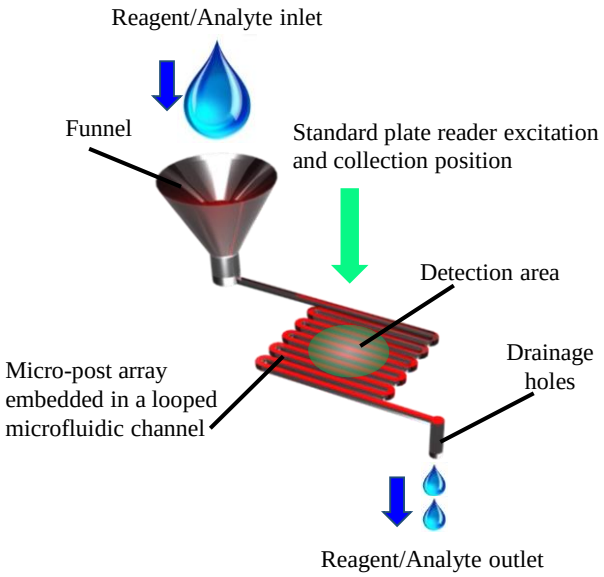


Figure 1. Working principle of a MicroFluere® well plate.

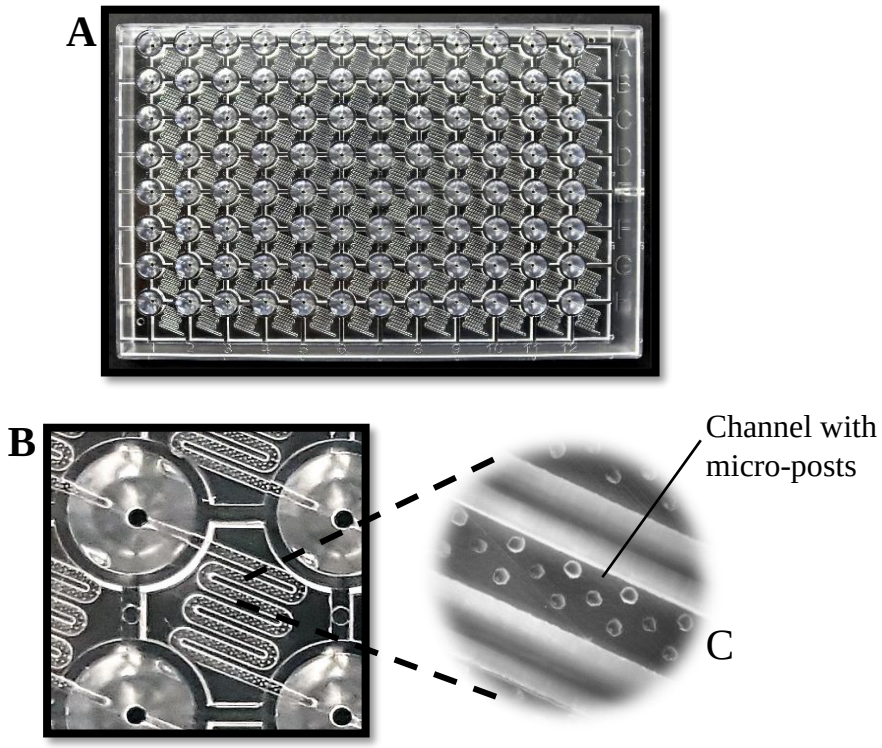


Figure 2. (A) A picture of MicroFluere® well plate. (B) Enlarged view at one of the microfluidic units. (C) Further zoomed-in picture of a microfluidic channel embedded with micro-posts.

In the looped microfluidic channel, an array of optically-transparent micro-posts are arranged in a symmetric pattern. The micro-posts extend perpendicularly from the top of the channel. The micro-posts are equally distributed throughout the entire detection area, and each micro-post has a generally cylindrical shape. The unique arrangement of the channel and the micro-posts within each microfluidic unit assures consistent optical measurements even in the presence of as large as 1 mm positive or negative lateral shift of the plate in the horizontal (X- axis) and/or vertical (Y-axis) direction with respect to the optical detection center, as exhibited in **Figure 3**. The results show that CVs (coefficient of variations) due to both X and Y movements are less than 5% (note: the plate reader itself may already cause 5% variation in optical signal reading).

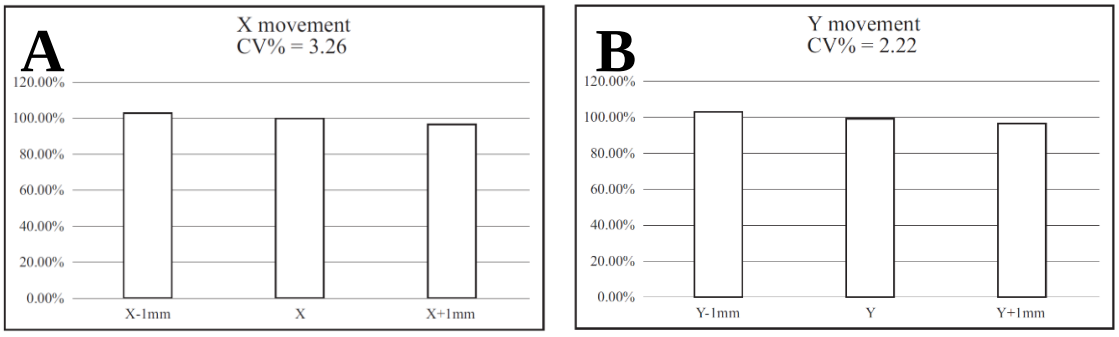


Figure 3. Fluorescence intensity from microfluidic units read by a standard plate reader at each specific X and Y position when each of the microfluidic units is filled with 10 µL of 5 µM Rhodamine 6G. (A) Relative lateral shift of +/- 1 mm in the X-axis. (B) Relative lateral shift of +/- 1 mm in the Y-axis.

Human Interleukin 6 (IL-6) ELISA in buffer and serum

As an example of the MicroFluere® plate performance, we tested various concentrations of Human IL-6 dissolved in buffer solution and serum with fluorescence detection method. MicroFluere® plates were validated by comparing results to traditional 96-well plates. A conventional ELISA protocol of R&D Systems (Kit #DY-206) was used for the traditional 96-well plate and the same protocol (but with less sample and reagent volume and shorter incubation times) was used for the MicroFluere® plate. The results of IL-6 in buffer and serum are shown in **Figure 4A** and **4B**, respectively. Both well plates have the same lower detection limit; however, the dynamic detection rage is further extended with the MicroFluere® plate. The advantages of MicroFluere® plates are summarized in **Table 1**.

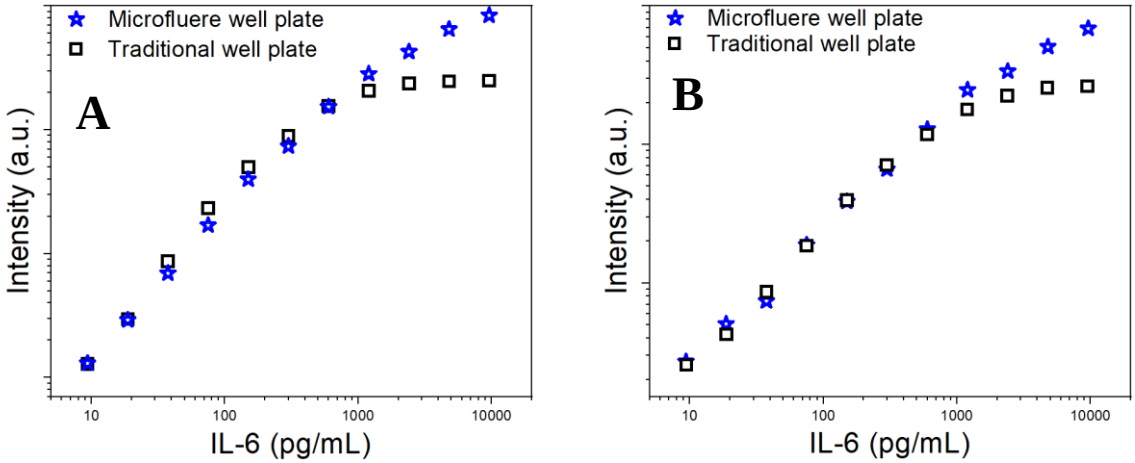


Figure 4. Comparison of IL-6 detection using a traditional 96-well plate (~300 minutes of assay time) and a MicroFluere® plate (<60 minutes of assay time). (A) IL-6 in buffer. (B) IL-6 in serum. The upper detection limit for the traditional and MicroFluere® plates are 1200 pg/mL and 9600 pg/mL, respectively. Note: since the readings for the traditional plate and the MicroFluere® are different, they are adjusted so that the readings for 9.6 pg/mL match.

Table 1. Comparison of human IL-6 ELISA using traditional and MicroFluere® well plates

	Traditional	**MicroFluere®**
Capture Antibody coating	Over night	1 hr
From adding analytes to recording results	~ 6 hrs	<1 hr
Limit of Detection (IL-6)	9.6 pg/mL	9.6 pg/mL
Dynamic range (IL-6)	9.6-1200 pg/mL	9.6-9600 pg/mL
Reagent/sample consumption	More than 100 µL	Less than 20 µL

Additional biomarkers testing results

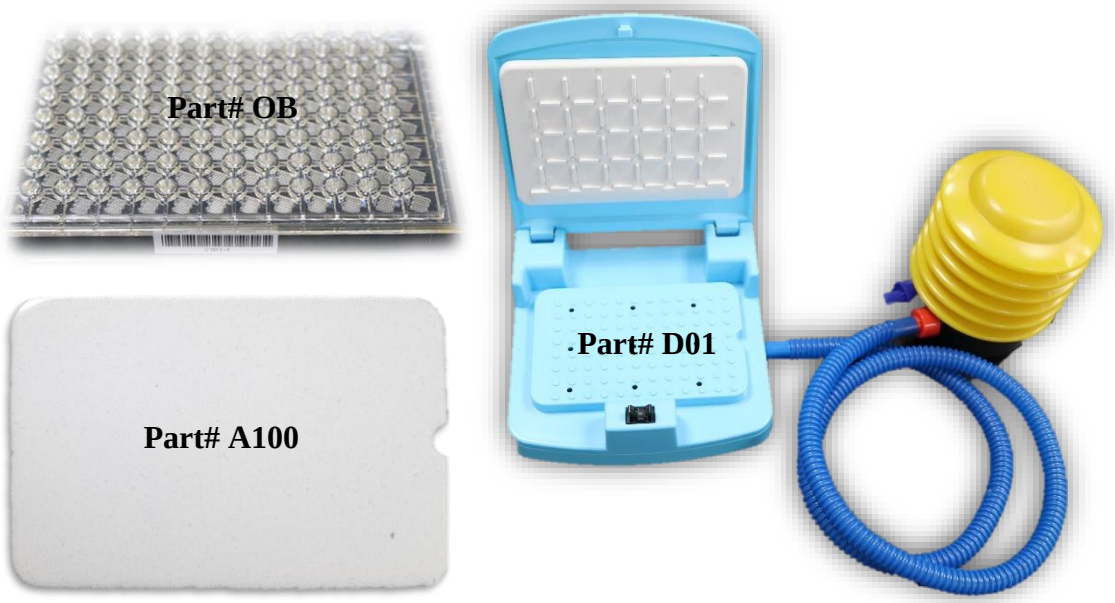
MicroFluere® well plates have been validated against traditional well plates with multiple biomarkers, including:

- Human IL-6 ELISA (R&D Systems DuoSet® kit # DY206)
- Human IL-2 (R&D Systems DuoSet® kit # DY202)
- Human IL-8/CXCL8 (R&D Systems DuoSet® kit # DY208)
- Human TNF-alpha (R&D Systems DuoSet® kit # DY210)
- Human IFN-gamma (R&D Systems DuoSet® kit # DY285)
- Human CXCL10/IP-10 (R&D Systems DuoSet® kit # DY266)
- Human VEGF (R&D Systems DuoSet® kit # DY293B)
- Human GDNF (R&D Systems DuoSet® kit # DY212)

The list is growing. In all of the above biomarkers (and kits), MicroFluere® plates have the same (or better) lower detection limits and larger dynamic ranges compared to traditional plates, while using only 20% of the sample/reagents and completing the assay 4-6X faster.

Product availability

We have launched MicroFluere® plates (Part# OB) and kit (Part# OBK) that includes drainage (Part# D01) device, and absorbent pads (Part# A100). Please contact us at info@optobio.com (Phone. +1-734-252-9645) if you would like to try free samples or buy small or large quantities.



Xpress ELISA

A

Fast, Affordable, Portable and Reliable Automated ELISA System

Xpress ELISA is a standalone desktop automated ELISA system and enables generation of results over 12X faster than standard ELISA while it needs less than 20 µL of sample.

Background

Current automated ELISA systems are generally not suited to deploy at point-of-care testing and other field applications due to bulky and expensive. In addition, it has such common drawbacks; long testing time, large sample and reagent consumption.

Optofluidic Bioassay, LLC has been developing a fast, affordable, portable, and reliable automated ELISA System- Xpress ELISA. It comprises a standalone desktop automated ELISA machine and disposable components. Overall size of the system is compact enough to operate on a regular desk and therefore it well-suited to point-of-care applications, emergency critical care sectors, as well as research and development laboratories and field applications where they have limited spaces. Recent results exhibited that it used only 8 µL of sample and produce result within 30 minutes which is over 12 times faster than traditional ELISA. Moreover, intra- and inter-assay variabilities are well below the traditional gold standard.

Technology

The Xpress ELISA system is an integration of automated ELISA machine and disposable components. The automated ELISA machine has four main components; (I) programmable robotic arm, (II) liquid handling module, (III) well place holder, and (IV) optical detection module as

shown in **Figure 1**. Estimated market price of the machine could be about $5,000 which is much cheaper than current ELISA automation systems in the market which is around $100,000. Disposable components include (A) disposal sensor array cartridge, and (B) disposable well plate which has reagent reservoirs and absorbent pads (See **Figure 1**).

The robotic arm is attached with liquid handling module that can connect to a disposal sensor array cartridge. ELISA reaction will be occurred inside of the cartridge as shown in **Figure 2**. Once the sensor array is connected, the movement of the robotic arm, and sequence of aspiration and dispensing of the liquid handling systems can be programmed based on desired protocol. In the disposable well plate, reagent reservoir (well B1) and absorbent pad (well B2) are alternately arranged as shown in **Figure 3**. Therefore, the sensor array cartridge can uptake reagents or sample from the reagent reservoir and dispose those into the absorbent pad. After completing sequence of the ELISA assay procedure, the sensor array cartridge is moving toward detection module and stations at designated position for optical detection. Finally, the optical module acquires an image and converted it to optical intensity for user. The duration of overall process is dictated by user's desired protocol. We have demonstrated assay time of 30 minutes for detection of follicle stimulating hormone (FSH) in serum and 40 minutes detection of HIV-1 Gag p24 in buffer.

Follicle stimulating hormone (FSH) ELISA in serum

In collaboration with the University of Michigan, we have published the experiments of follicle stimulating hormone (FSH) detection using our Xpress ELISA system compared with standard traditional method in ACS Sensors journal. The standard method for mouse FSH (mFSH) quantification usually takes 2 days and needs lager sample volume of 60 μL. With our Xpress ELISA system, highly sensitive quantification of mFSH can be accomplished within 30 min using only 8 μL of the serum sample. Experimental detail can be found in the ACS Sens., 2018, 3 (11), pp 2327–2334 (DOI: 10.1021/acssensors.8b00641) that published October 18, 2018 by American Chemical Society. (web https://pubs.acs.org/doi/10.1021/acssensors.8b00641)

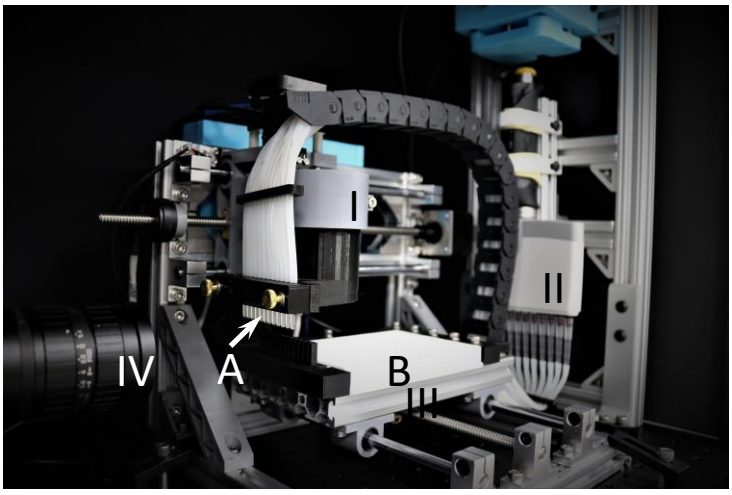


Figure 1. A photograph of a functional prototype of Xpress ELISA system

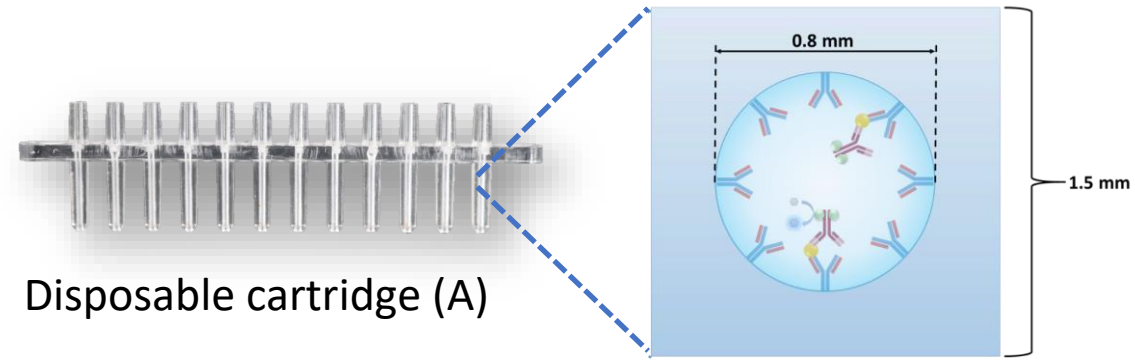


Figure 2. A photograph of a disposable sensor array cartridge (left) and a schematic of ELISA reaction in a capillary of the cartridge (right)

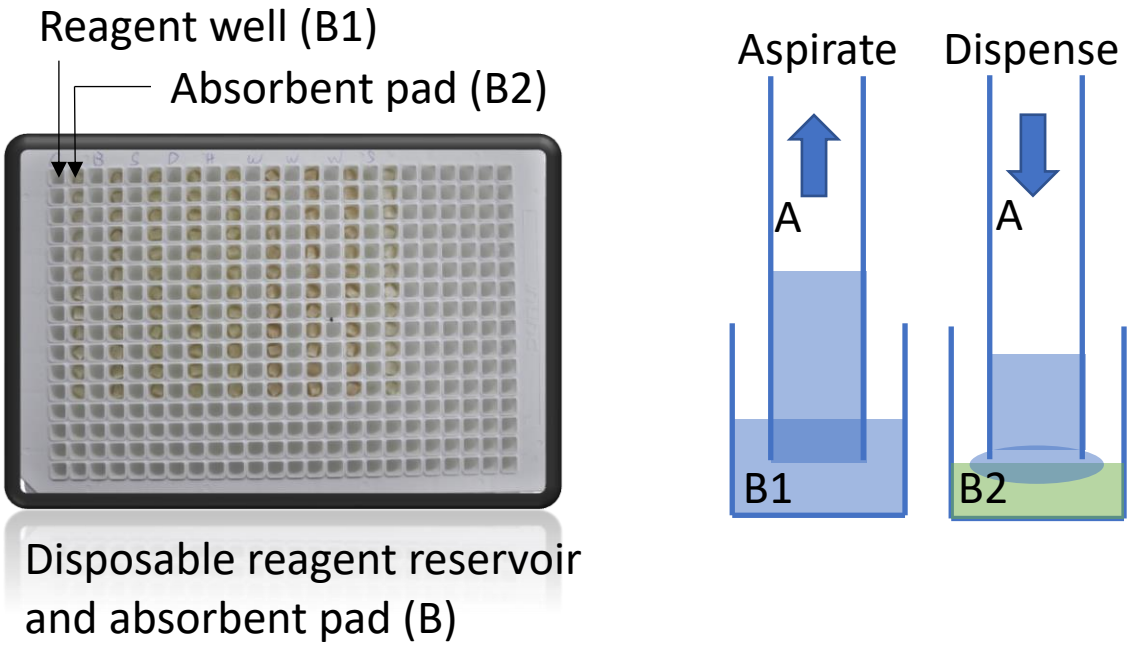


Figure 3. A photograph of a well plate containing reagent reservoirs and absorbent pads (left) and a schematic of aspirate and dispense mechanism (right)

HIV-1 Gag p24 ELISA in buffer

Simultaneously, we evaluated intra- and inter-assay variabilities of the Xpress ELISA using HIV-1 Gag p24 (R&D Systems Kit #DY7360-05) biomarker in buffer as a sample analyte. The system can produce the result in 40 minutes after adding sample. We tested two sperate Xpress ELISA systems with two different sensor cartridges. In each cartridge, two sensors were loaded with the same concentration of the biomarker. The data were plotted against concentrations of the biomarker (**Figure 4**). By using 4 parameter logistic regression (4PL) curve fit in those data, the coefficients of determination (R squared) of both measurements are extremely close to 1 (0.99957 and 0.99939). That indicates prediction of un-known concentration of analyte within the detection range will be very accurate. Furthermore, coefficient of variations of the system are excellent and well below the acceptable

standard ELISA requirements of 15% in intra-assay and 20% in inter-assay as shown in **Table 1**. Therefore, the system empowers to produce high precision results.

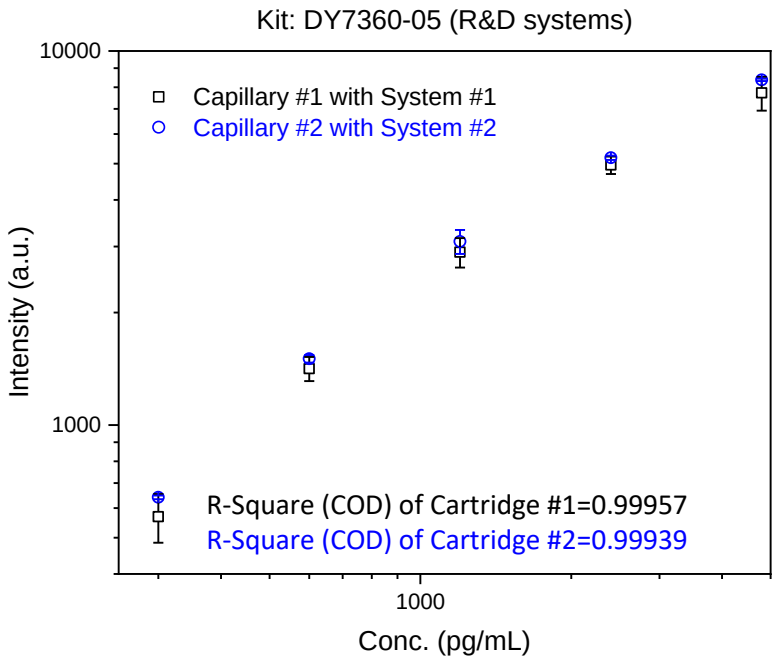


Figure 4. A standard curve of HIV-1 Gag p24 produced by two separate Xpress ELISA systems with two different sensor array cartridges within 40 minutes of assay time

Table 1. Intra- and inter-assay variabilities of HIV-1 Gag p24 ELISA using Xpress ELISA system

Concentration (pg/mL)	**300**	**600**	**1200**	**2400**	**4800**
Intra-assay variability (CV of Cartridge #1)	6%	1%	1%	2%	7%
Intra-assay variability (CV of Cartridge #2)	1%	2%	7%	1%	1%
Inter-assay variability (CV of Cartridge #1 and #2)	15%	7%	9%	5%	10%

Product availability

We have completed functional protype and evaluated it as described above. We are developing market ready product and anticipate that the product will be available in market by 2020. If you are interested in this product, please contact us at info@optobio.com (Phone. +1-734-252-9645).

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