# Development of a microfluidic circuit with femtosecond laser technology for enzyme-linked immunosorbent assay validation.

Cristhiano C. Herrera, Martha S. Ribeiro, Patrick J. Spencer, Ricardo E. Samad; Nilson D. Vieira Jr, Wagner de Rossi

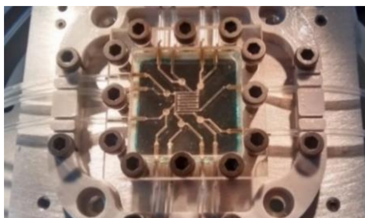
cristhianoherrera@usp.br; marthasr@usp.br; pspencer@ipen.br; resamad@ipen.br; nilsondv@ipen.br; wderossi@ipen.br

**Abstract:** *One of the great challenges in science today is to develop applications for disease diagnosis that can be accessible to the poorest populations distant from large centers. Using ultra-short laser pulses, we developed and machined a microfluidic circuit capable of replicating enzyme-linked immunosorbent assays (ELISA) with a much lower cost of materials. Microchannels etched in BK7 glass separated by a PDMS film form the basis of the system. The reagent flow control is done by pneumatic micro-valves controlled by an Arduino microcontroller through a Labview platform. The enzymatic reactions occurred under stopped-flow conditions, in a portion of the circuit constructed in a serpentine shape to facilitate the detection by an image processing software. This work presents the design of the microfluidic circuit developed and an ELISA test for characterization and validation of the results.*

**Key-Words:** *microchannels; PDMS; BK7; enzyme-linked immunosorbent assays; femtosecond machining*

**Introduction:** The field of microfluidics has encouraged the development of miniaturized laboratory equipment and processes. These processes proffer minor reagent consumption, automation, compactness, and cost effectiveness. The experimentations duration may be drastically reduced, from hours for typical immunoassays realized in microtiter plates, to minutes with microchannels [1]. The introduction of enzymes as antibody markers opened new possibilities for antiglobulin tests in serology by originating practical tests for routine purposes, such as the enzyme-linked immunosorbent assay (ELISA)[2]. We developed a microfluidic circuit for ELISA test with automated flow control to reduce reagent volumes and reaction times, and described a Jararaca antigen test that shows the efficiency of the system.

**Experimental:**

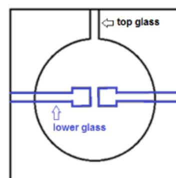


The microfluidic circuit machined for the ELISA test consists of 6 inputs and 1 output for fluids, as shown in the figure on the left.

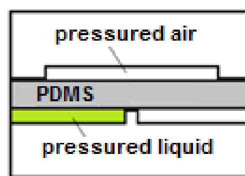
The flow of the reagents is controlled by the activation of pneumatic microvalves allowing or not the flow of the reagent to the serpentine region where the reaction occurs.

Microcontrolled switchings allow flows ranging from tens of nanoliters to hundreds of microliters.

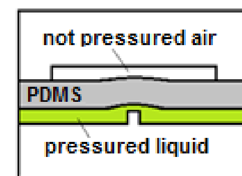
The scheme illustrates the flow control:




Upper view



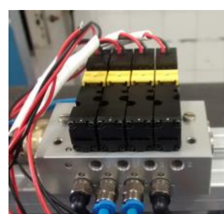

Closed valve




Open valve

The fluids used in the reactions are stored in syringes and pushed with 8 PSI of pressure through microchannels built into the lower glass slab, while pneumatic valves provide 14 PSI of pressure through the microchannels etched into the top glass slab. By controlling the removal of pneumatic pressure in these upper channels through the Arduino microcontroller it is possible to control the flow of the reagents individually.

The figures below show the pneumatic valves and the reagent reservoir system.



The following reagents were placed in the syringes:

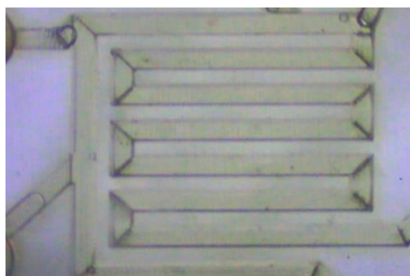
| Syringe | Reagents |
|---|---|
| 1 | Jararaca antigen (100 ug / ml diluted in TBS) |
| 2 | TBS - tris-buffered saline, pH 8 (wash solution) |
| 3 | 3% skim milk diluted in TBS (blocking solution) |
| 4 | Antibotropic serum of Butantan, 1: 5000 in TBS (primary antibody) |
| 5 | IgG anti-horse IgG (whole molecule)-peroxidase antibody, 1:5000 (secondary antibody) |
| 6 | orthophenylenediamine (1mg/ml in TBS with 0.15% $H_2O_2$) |

Process:
- The circuit was sensitized overnight with the jararaca antigen (1).
- The micro reactor was then washed with the TBS (2) solution to remove non-adsorbed antigen.
- The blocking solution (3) was injected in the reactor with the purpose of adhering in spaces in which the antigen did not adhere.
- The circuit was washed with TBS solution (2) for 4× to remove non-adhered milk.
- The next step was the inoculation of primary antibody (4) that remained in the circuit for 40 min and was then washed with TBS (2) for 4×.
- Then, the secondary antibody (5), which is responsible for the reaction, was placed and remained incubated in the loop for 40 min. After this time, was washed with TBS (2) 4x.
- In order to induce the colorimetric reaction the orthophenylenediamine solution (6) was injected into the loop and the color change (transparent to yellowish) was observed in approximately 20 min.

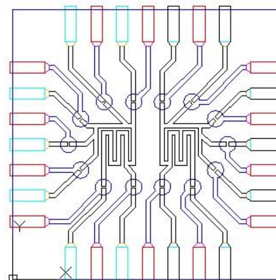
Note: The same procedure was done in a 96-microtiter plate (100 µl) with and without antigen in the same amounts for false positive verification.

**Results and discussion:** The results obtained in both the 96-microtiter plate and the microfluidic circuit are similar. However, the amount of reagents used in the microfluidic circuit was much lower allowing a significant reduction in costs. The automation of the process in the microfluidic system allows a more effective control and avoids contact with reagents that can occur in the manual process of the 96-microtiter plate. The processing time of the tests were the same, but studies are in progress to reduce these times since reactions in microchannels occur in a much shorter time.

The figure below shows the change from transparent to yellow color denoting the presence of the antigen.



A new microfluidic system with two similar circuits is being developed for antigen detection and false positive verification, giving more assurance to the test.



The same microfluidic system can be used for different detections and we are initiating tests to detect *Toxoplasma gondii* antibodies in blood serum for Toxoplasmosis diagnosis, a risky disease especially for pregnant women.

**Conclusion:** Enzyme-Linked Immunosorbent Assays (ELISA) are perfectly feasible in microfluidic systems, reducing the reagents volume and their cost, and the detection of Jararaca antigen was used as proof of concept.

**References and acknowledgements:**


[1] DUBUC, D., CHEN, T., CHRÉTIENNOT, T., POUPOT, M., FOURNIÉ, J. Microfluidic On-Chip for Biomedical Applications. ***IEEE***, p. 129-132, 2011.

[2] CAMARGO, M.E., FERREIRA, A.W., MINEO, J.R., TAKIGUTI, C.K., NAKAHARA, O.S. Immunoglobulin G and immunoglobulin M enzyme-linked immunosorbent assays and defined toxoplasmosis serological patterns. ***American Society for Microbiology - Infection and Immunity***, v. 21, p. 55-58, 1978.