

# LAB-ON-A-CHIP FOR LABEL-FREE MULTI-DETECTION OF RESIDUAL ANTIBIOTICS IN MILK

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## ABSTRACT

This paper reports the design, fabrication and test of microfluidic cartridges that integrate reservoirs, channels, incubation chamber and optical transducer for automated multi-antibiotics detection in raw milk. The resulting lab-on-a-chip is pre-filled with the biological solutions required for the immunoassay. The whole detection sequence is operated automatically by external pump and valves combination. The immunoassay principle is based on a competitive assay format, and the resulting refractive index changes at the sensor surface is measured by wavelength interrogated optical sensing (WIOS) method. Using the developed instrument, two antibiotic families were simultaneously detected in less than 10 minutes.

**KEYWORDS:** antibiotics detection, milk, optical sensor, WIOS

## INTRODUCTION

Today, strict regulations for food safety have been established. Concerning the quality of milk, Maximum Residues Limits (MRL) for antibiotics have been defined in the European Union legislation. That has led the development of accurate, fast, and cheap tests for the antibiotics detection in milk such as dip stick [1] tests. However, they are not fully automated and more over, they are unable to detect more than two antibiotics in one single analysis.

The goal of our research was to develop a lab-on-a-chip (Fig.1) for simultaneous multi-antibiotics detection in raw milk. In comparison to the above mentioned dip stick tests, the presented lab-on-a-chip is able to detect multiple antibiotics simultaneously with fully automated sequence.

## WORKING PRINCIPLES

The detection principle is based on wavelength interrogated optical sensing (WIOS) [2] that detects the refractive index changes in the evanescent wave of a straight waveguide grating using a wavelength sweep. The sensing chip is constituted of eight individual sensing regions (grating pads) that allow simultaneous and real-time monitoring.

We use competitive immunoassay formats in order to reach low limits of detection (Fig.2). Sensor chips are biofunctionalized with different biorecognition molecules (haptens and receptors) that exhibit high specificity towards their analytes.

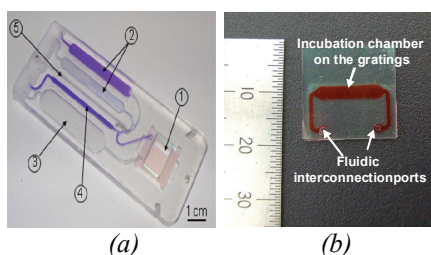
The reservoirs integrated in the chip are pre-filled with biological solutions. Fluids motion is driven by a single syringe pump working in aspiration mode in combination with a multiposition valve that selectively connect the reservoir to atmosphere. To remove the residual air between solutions, the liquid goes first to the loading waste and then the flow deviates towards the sensing chamber for reaction/measurement. Milk sample introduction is ensured through external reservoir (septum-vial) connected to cartridge and valve. Waste reservoirs ensure that no residual liquid is going out of the cartridge (no contamination).

## FABRICATION

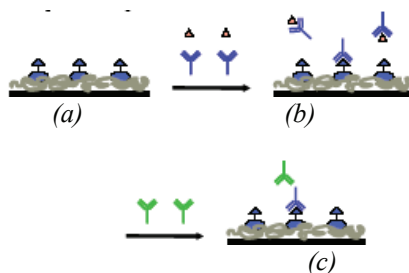
A hybrid type cartridge (Fig.1a) was developed based on PMMA with micromilled channels and reservoirs of 100  $\mu\text{L}$  combined with HBP (hyperbranched polymer) [3,4] incubation chamber. The HBP used in this work is an acrylated, second generation polyether core HBP. It combines a high Young's modulus, a hydrophilic character and low internal stresses resulting in high dimensional accuracy. A novel fabrication technique combining UV micromolding and photolithography allowed the fabrication of microfluidics components and interconnection port simultaneously, as shown in Fig.3. The incubation chamber in hybrid type cartridge fabricated by the process described in Fig.3 is shown in Fig.1b.

## RESULTS AND DISCUSSION

The full immunoassay sequence was automatically operated with no air entrapped in incubation chamber. Figure 4 shows immunoassay WIOS response obtained from the hybrid type cartridge was for the detection of two antibiotic families (sulfonamides and fluoroquinolones). Within 10 minutes from the sample introduction, a clear signal differentiation is observed between reference and biofunctionalized channels. High differential response values obtained at MRL concentration (100  $\mu\text{g}/\text{kg}$ ) allow binary response test.



*Fig.1. The lab-on-a-chip for detection of antibiotics in milk: (a) micromilled PMMA cartridge combined with sensor chip: (1) sensor chip; (2) reservoirs for biological solutions; (3) main waste; (4) loading waste; (5) sample introduction channel; (b) HBP incubation chamber bonded on the sensor chip (1 of Fig.1a).*



*Fig.2. Immunoassay protocol: (a) bio-functionalization of sensor surface; (b) competition step; (c) amplification.*

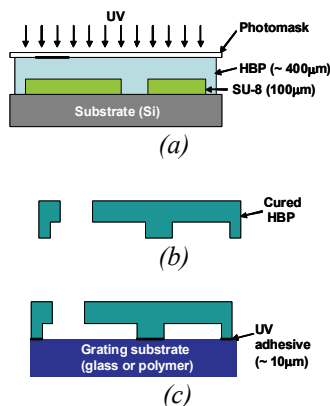


Fig.3. Fabrication process of the HBP microfluidics: (a) UV curing of HBP on SU8 through photomask; (b) development of uncured HBP; (c) bonding on the grating substrate using UV adhesive.

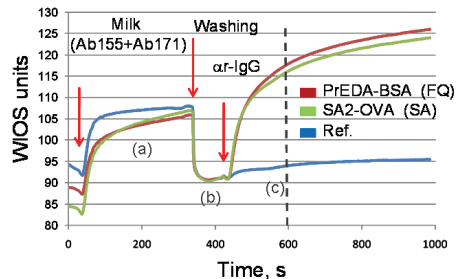


Fig.4. Lab-on-a-chip immunoassay response for two antibiotics detection: (a) milk sample with competitive antibodies; (b) washing with buffer solution; (c) amplification with secondary antibody. Blue line: reference (non-specific surface); Green line: sulfonamides hapten; Red line: fluoroquinolones hapten.

Ongoing work is devoted to the development of a miniaturized on-chip cartridge based on reservoirs of 10 µL and microchannels in a single chip and to detect four antibiotics families.

## CONCLUSIONS

A fully automated lab-on-a-chip for simultaneous detection of multi-antibiotics in raw milk has been developed. The microchannels, reservoirs and incubation chamber are integrated in the chip using a combination of micromachining and micromolding based on low-stress HBP material, resulting in cheap and fast test. The preliminary results are promising for fast binary test. Lab-on-a-chip is currently being upgraded for detection of four antibiotic families.

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## REFERENCES

- [1] <http://www.twinsensor.com>
- [2] K. Cottier, M. Wiki, G. Voirin, H. Gao and R. E. Kunz, *Sens. Actuat. B*, **91**, 241.
- [3] L.E. Schmidt, D. Schmaeh, Y. Leterrier, and J.-A.E. Månson, *Rheologica. Acta.*, **46**, 693.
- [4] Y.-H. Jin, Y.-H. Cho, L.E. Schmidt, Y. Leterrier, and J.-A.E. Månson, *J. Micro-mech. Microeng.*, **17**, 1174.