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Laboratory skinpatches and smart cards based on foils

Jesús M. Ruano-López, Florian Laouenan, Maria Agirregabiria, Sonia Rodriguez, Jan Krüger, Diarmuid Flavin

Abstract—**This paper presents: (i) a hand held system consisting of a portable platform and disposable polymeric Lab-on-a-card capable of performing a nucleic acid concentration, amplification and detection with reagents inside; and a (ii) wearable diagnostic Lab-on-a-Paper skinpatch system, capable of performing in-situ sweat sampling, analyte pre-concentration, and immunoassay analysis. The skinpatch works in conjunction with a hand-held optoelectronic reader / micro PC fluorescence analysis interface.**

I. Introduction

Miniaturisation of biological assays in Lab on a Chips (LOCs) has very well known advantages[1,2]. However, it still needs to select the target molecules out of a large complex sample and placed them precisely on top of the biosensor area[3]. In the particular case of sample preparation and Polymerase Chain Reaction (PCR), Kim et *al.*[4] fabricated a Polymethylmethacrylate (PMMA) microchip where PCR reagents were stored under a paraffin layer in the same chamber where the amplification took place. Gulliksen *et al*[5].presented a Cyclo Olefin Copolymer (COC) LOC where RNA purification and a nucleic acid sequence-based amplification (NASBA) was carried out by injecting a crude *E. coli* cultured lysate. El-Ali *et al.* performed Cell dielectrophoresis and PCR in a SU8 chip with a PDMS cover[6]. Agirregabiria *et al.* developed a DNA sample preparation and PCR on a SU8-one-chamber chip for gram positive bacteria such as Salmonella *spp.* in human faeces.[7] This chip design was further used to detect Campylobacter *spp.* in chicken faeces by PCR[8] and identify influenza viruses in nasopharyngeal human sample by Retro Transcriptase qPCR[9]. More recently, Lee *et al.* developed a similar LOC together with its reader for gram negative bacteria[10].

This paper shows a portable system and nucleic acid LOC in the form of a laboratory smart card (Labcard). Unlike previous works: (i) The sealing process is carried out at a low temperature by a pressure sensitive tape avoiding any damage of the previously stored PCR reagents; (ii) The use of integrated valves in the Labcard; and (iii) The complex sample preparation, transport of the eluted DNA and qPCR are carried out in an automatic manner without the user intervention.

The Lab-on-a-Paper analysis system comprises a disposable diagnostic test device, which can be worn on the skin and is designed to analyze human sweat for the presence of Cocaine. The skinpatch's analytical method is based on immunochromatographic assays and laser induced fluorescence detection (LIF).

II. DESIGN

A. Labcard Design

The Labcard comprises two inlets, two outlets, five microvalves, and two chambers (a concentration chamber and an amplification chamber with stored reagents) (Figure 2). It is made of a COC substrate sealed by a polypropylene film coated with a pressure sensitive adhesive. The assay is carried out in a sequential manner and automatically controlled by a laptop. First, magnetic bead-based concentration, washing and elution are carried out in the first chamber. Then, the eluted DNA is transferred to the second chamber where the stored reagents are rehydrated. Finally, a quantitative PCR (qPCR) takes place in the second chamber. The fluorescence created during the qPCR is recorded in real time by the platform. The use of the magnetic bead protocol allows the Labcard to process a wide range of sample volumes (from 10 µl to 10 ml). The reagents are stored inside the labcard allowing long-term storage, reduction of reagent volumes, simplification of the Labcard design, and finally easier automatisation. The cross contamination is avoided by making the Labcard disposable. The low cost labcard is guaranteed by a demonstrated mass production process and by limiting the integration of components within the Labcard. Hence, the portable platform keeps all the costly components outside the labcard: an external fluorescence detector, a peristaltic micropump, two heaters and their temperature sensors, two magnets and 5 pin actuated micromotors. This platform is connected to a laptop PC by an Universal Serial Bus (USB) connector where the final result is displayed in the form of a typical realtime curve (Fig. 1).

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J. R., M. A., F. L., and J.B. authors are at Ikerlan-IK4, Arrasate, Spain, (e-mail:jmruano@ikerlan.es)

S. R. and G. G. authors are at Biotools B&M Labs., Madrid, Spain.

M.S. author is at Fraunhofer Institute for Photonic Microsystems (IPMS) Dresden, Germany.

R.W. author is at Politechnika Wrocławska (WEMiF), Wrocław, Poland.

J. K. and D. F authors are at Biosensia, Dublin, Ireland.

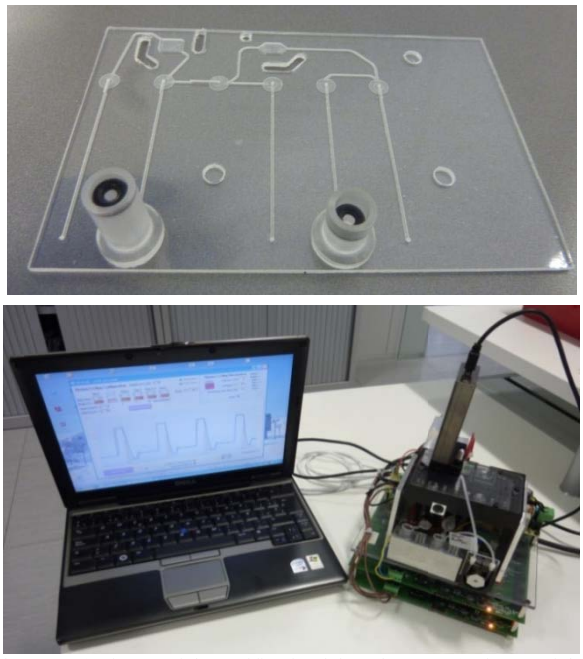

Fig. 1. Top) Picture of the verification labcard, Bottom) Picture of the developed labcard platform with the needed actuators for automatic sample.

B. *Skinpatch design*

The skinpatch device is single use test device made of adhesive polymers and integrated sweat sampling and immunoassay components (see Fig. 2). The device is designed for an operational lifetime of 1-5 days during which it will be worn on the skin of a patient or test person. For these reasons it is small in size, unobtrusive and light weight in order to be worn on person's skin with little or no discomfort.

The skinpatch uses dried immunoassay components in a lateral flow format. The test method is based on a competitive immunoassay approach, whereby the analyte (Cocaine antigen) competes with a fluorescently labelled conjugate of the drug to bind to immobilized benzoylecgonine (Cocaine metabolite).

The skin patch is an entirely passive device: 1) the sample collection and pre-concentration takes place in-situ while the skinpatch is worn by the patient or test person for up to 5 days; 2) the immunochromatographic reactions take place after the wearing period and are initiated through manual injection of fluid reagents into the skinpatch device; 3) the skinpatch analysis takes place with an external fluorescent reader, which simultaneously excites and detects emission from the fluorescently conjugated antibodies. The reader software compares the detected emission peaks with pre-calibrated data and generates a fail or pass output signal.

III. Experiment Description

A. *LabcardReader*

The reader has the labcard holder with one pipette inlet (sample) and three inlets/outlets for automatic injection of the sample, washing buffer and elution buffer from eppendorf tubes. The reader also has: two heaters, one for each chamber; two magnets on each side of the concentration chamber; and 5 micromotors to bend the 5 membrane valves inside the Labcard. The fluorescence detector is a commercial device capable of detect two fluorophores (ESElog, ESE, Germany). In order to pump the sample and reagents into the labcard a small peristaltic pump (Model P625, Instech, USA) is used. The sample is injected by placing the peristaltic tube inside the sample eppendorf. Similarly, the elution buffer is injected by a second peristaltic tube placed by hand inside the eppendorf that contains this buffer. This platform is connected to a dedicated Printed Circuit Board that is connected by an USB to a laptop where a software interface controls the fluidic injection, valves, heaters and optical detector.

B. *Skinpatch Reader*

The skin patch co-works with a hand-held optoelectronic reader connected by a USB port to a portable personal computer (Fig. 2). Recent developments in optoelectronics – including OLED, laser and CCD matrix – and software enhanced analyze of the fluorescence readout are involved in the hand-held reader.

Fig. 2. Pictures of the developed labonapaper (left) and its reader (right).

IV. Results

A. *Labcard experiments*

The carried out experiment consisted of the following steps represented in Fig. 3.

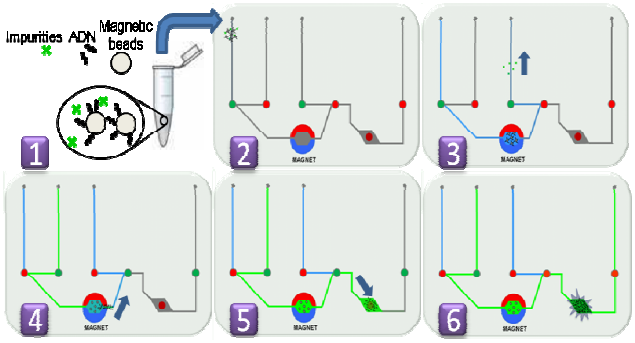

Fig. 3. Schematic representation of the carried out steps.

Briefly, the steps are:

1) 5µL of DNA, 10µL of magnetic beads, 50µL of isopropyl alcohol are added to 995µL of nuclease-free water.
2) The DNA concentration is done by magnetic separation using two magnets placed on both sides of the first reaction chamber.

3) As the sample containing the magnetic beads and DNA passes through the chamber, the beads are retained in the magnetic field generated by the two magnets. The impurities leave the labcards and only the beads and bound DNA remain inside the chamber.
4) The DNA is eluted using elution buffer injected and carefully transferred to the PCR chamber.
5) The DNA and PCR reagents are mixed rehydrating the stored reagents.
6) Once the amplification chamber is completely filled with eluted DNA, the appropriate valves are closed and the thermocycling begins with the fluorescence detector activated.

The following graph shows a result where nucleic acid concentration, amplification and detection took place with reagents stored in the Labcard.

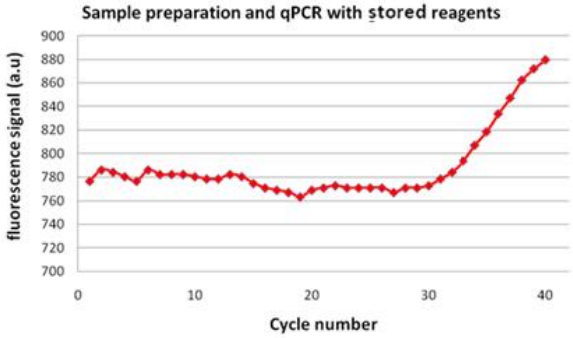

Fig. 4. Final result once the nucleic acid concentration, amplification and detection took place with reagents stored in the Labcard.

B. *Skinpatch experiments*

At the current stage of the pre-validation laboratory procedure, the detection limit of cocaine is significantly improved to level of a few ng/ml (Fig. 5) in comparison to existing strip tests where 300 ng/ml limit is now a standard. What more, quantitative detection of cocaine, in spite of only indication of presence of the cocaine in a sample as it is now, is possible.

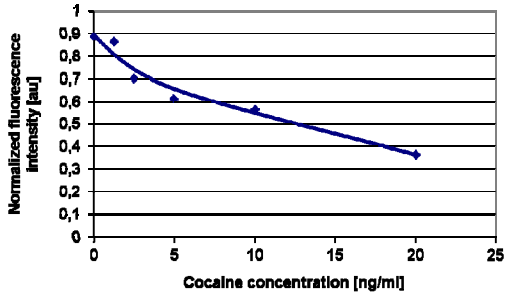

Fig. 5. Normalized fluorescence intensity of the test line as function of cocaine concentration in the artificial human's sweat containing sample

V. CONCLUSION

This platform with a Labcard with two chamber strategy, integrated valves and stored PCR reagents ensure (i) compatibility with complex sample by removal of PCR inhibitors from the crude sample, (ii) automatic injection of reagents by the actuation of integrated valves, (iii) long shelf-life or uncontrolled temperature transportation of the Labcards by storing the PCR reagents and (iv) disposability of the LOC due to its simple and inexpensive mass fabrication compatibility (injection molding and lamination).

Furthermore, the range of future applications of this Labcard is wide since the sample used in this paper is compatible with a extremely simple sample preparation off the LOC, just mixing the sample (a swab, sputum, blood) with a mixture that contains a lysis buffer and magnetic beads similarly as it was done in previous authors works [7,8,9]. Afterwards, this tube can be injected in the Labcard as explained before. In fact, this work is framed within an European project where this Labcards will be adapted for CEA protein detection by Immuno-qPCR, food pathogen typing by multiplex PCR and sea algae detection by NASBA.

At the current stage of the LABONFOIL project, the SkinPatch application is dedicated for detection of cocaine. However, it can be easily tailored to other applications by change of the biochemical part of the patch toward highly sensitive and cheap test patch co-working with low-cost optoelectronic reader.

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