

DNA concentration, elution, and real-time PCR amplification inside a Labcard platform

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We present a hand held system consisting of a portable platform and disposable Labcard capable of performing a nucleic acid concentration, amplification and detection (Figure 1). 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 Cyclo Olefin Copolymer (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 Polymerase Chain Reaction (qPCR) takes place in the second chamber. The fluorescence created during the qPCR is recorded in real time³ 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 automatization. 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 (Figure 3).

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 area³. In the particular case of sample preparation and 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 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 cover⁶. 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 PCR⁸ and identify influenza viruses in nasopharyngeal human sample by Retro Transcriptase qPCR⁹. More recently, Lee *et al.* developed a similar LOC together with its reader for gram negative bacteria¹⁰.

¹ C. Zhang, J. Xu, W. Ma, W. Zheng, *Biotechnology Advances* (2006), **24** (3), pp. 243-284.

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³ J. Kim, D. Byun, M.G. Mauk, H.H. Bau, *Lab on a Chip* (2009), **9** (4), pp. 606-612.

⁴ J.M. Ruano-López, *Lab on a Chip* (2009), **9**(11), 1495-1499.

⁵ A. Gulliksen, F. Karlsen, H. Rogne, E. Hovig, R. Sirevåg, *Lab on a Chip* (2005), **5** (4), pp. 416-420.

⁶ J. El-Ali, I. R. Perch, C. R. Poulsen, M. Jensen, P. Telleman and A. Wolff, *Transducers*,(2003) pp 214, USA.

⁷ M. Agirregabiria, D. Verdoy, G. Olabarria, P. Aldamiz-Echevarría, J.M. Ruano-López, *MicroTAS 2007*, France.

⁸ D. Bang, M. Agirregabiria, R. Walczak, J.A. Dziuban, A. Wolff, J.M. Ruano-Lopez, *MicroTAS* (2009), Korea.

⁹ D. Verdoy, Z. Barrenetxea, L. Fernández, M. Agirregabiria, J. Berganzo., J.M. Ruano-López, J.M. Marimón, M. Montes M., S. Hammoumi, E. Albina, G.Olabarria, *MicroTAS 2009*, Jeju, Korea.

¹⁰ Jeong-Gun Lee, K.H. Cheong, N.M. Huh, S. Kim, J.W. Choi and C. Ko, *Lab on a Chip* (2006), **6** (7), pp. 886

This paper shows a portable system and nucleic acid LOC in the form of a 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.

Figures

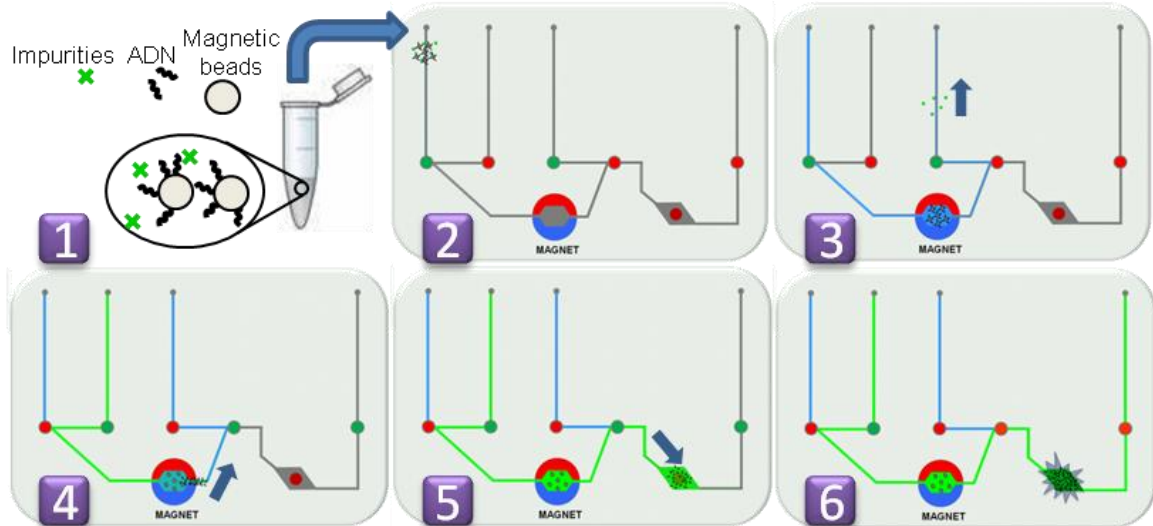


Fig. 1. 1) 5 μ L of DNA, 10 μ L of magnetic beads, 50 μ L of isopropyl alcohol are added to 995 μ L of nuclease-free water; 2) The DNA concentration is done by magnetic separation using two magnets placed on both sides of the first reaction chamber; 3) As the sample containing the magnetic beads and 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.

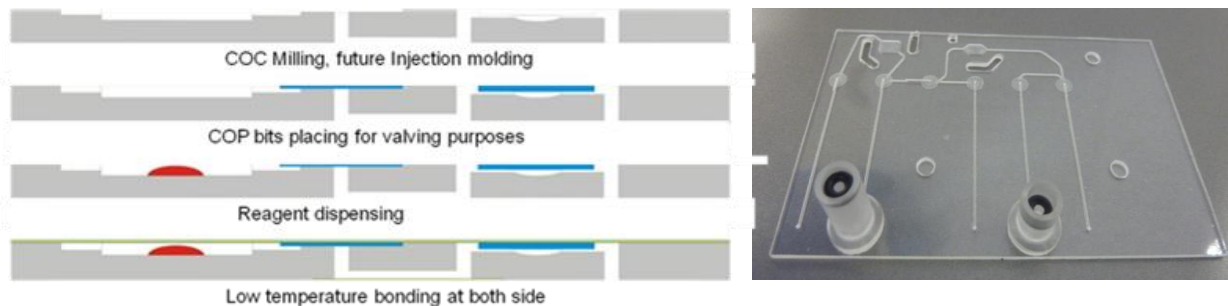


Fig. 2: Left) Schematic representation of the fabrication steps. Right) Picture of the verification labcard.

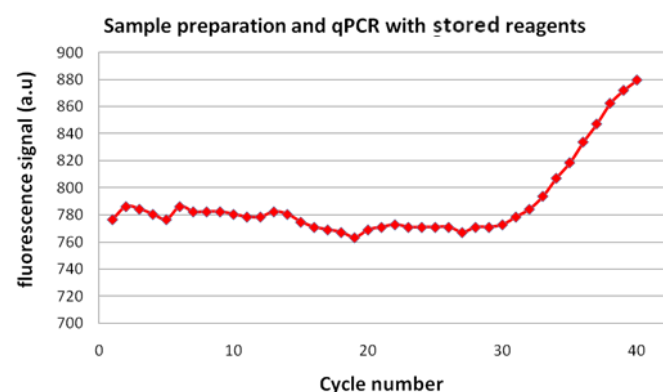
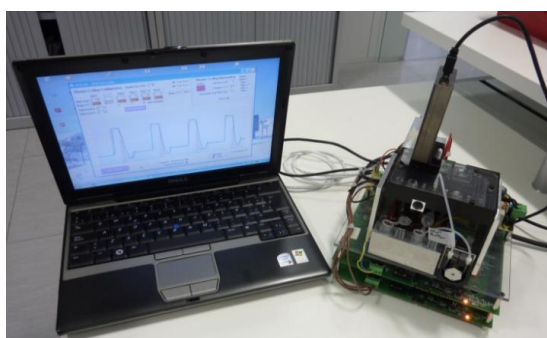


Fig. 3. Left): Picture of the developed labcard platform. **Right)** Graph showing the final result once the nucleic acid concentration, amplification and detection took place.