ANGELAB, EU project to develop Non Invasive Prenatal Diagnostics systems based on a modular LabonaChip format

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Summary

This paper reports a novel IVD system where Non Invasive Prenatal Diagnostic (NIPD) in-tube methods have been transferred to a LabonaChip format. The system uses a LabonaChip to carry out the whole analysis process: from total free DNA extraction from plasma to known mutation detection by qPCR.

Thanks to the used method and architecture, the development of a microfluidic-based IVD system is simplified. Additionally, the concept of modularity provides to the product a high level of flexibility. It enables to generate on-demand LOC-based IVD systems. This paper also presents results of three developed Non Invasive Prenatal Diagnosis systems using mother blood such as aneuploidies but also, a known mutation associated to monogenic diseases as Cystic Fibrosis (CF), and X-linked disorders.

Motivation and results

Existing gold standards for fetal genetic diagnosis are invasive techniques, which are risky and expensive. On the other hand current non invasive alternatives have low sensitivities and specificities.

All LabonaChips have been conceived with a modular architecture of different chip units, which are repeated in 8 lines, providing different valves, inlets/oulets, concentration and heating chambers (see Figure 1-3).

For the mutation detection, the protocol consists of three steps: i) tfDNA extraction from 800 µl of plasma from pregnant women, ii) tfDNA digestion using methylation sensitive restriction enzymes, and finally, iii) qPCR detection of target mutations. TfDNA extraction was demonstrated in-chip (Figure 4), starting from plasma from pregnant women carrying male fetuses. Figure 5 shows the reliability of the extraction in chip. Digestion in chip was also demonstrated. tfDNA extracted from pregnant plasma in tube was mixed with *BstUI* enzyme and incubated at 60ºC inside the microchamber. Figure 6 shows the comparison between pre-digestion post-digestion samples. Finally, qPCR was performed in chip to demonstrate the capability to distinguish between wild type, heterozygous and homozygous mutated p.Phe508del state corresponding to CF disease. DNA sample was divided in two identical aliquots to carry out a duplex qPCR in each of the two parallel microchambers. Same primers but different MGB probes labeled with FAM (Wild Type) and VIC (mutated) were used. Figure 7 shows the curves obtained in chip and in tube. Ct values were very similar.

The detection of this know mutation has been also demonstrated through a novel digital PCR technique developed within this work.

This paper will also reports a Lab on a Chip (LOC) system developed for Methylated DNA Immunoprecipitation (MeDIP), washing and pre-concentration of free fetal DNA (ffDNA) from maternal plasma for non invasive prenatal diagnosis (NIPD) of Trisomy 21 (Figure 8). After MeDIP, the Methylated DNA bound to magnetic beads is transferred to the next chamber at a flow rate of 30 µL/min, where beads are captured using a cylindrical NdFeB magnet 3 mm in diameter and 3 mm high. Washing is carried out into the same chamber with IP buffer in a continuous way keeping the beads captured.

In summary, complex LOC systems comprising both a microfluidic devices and an automatic Control Units have been developed for fetal DNA diagnosis from maternal plasma.

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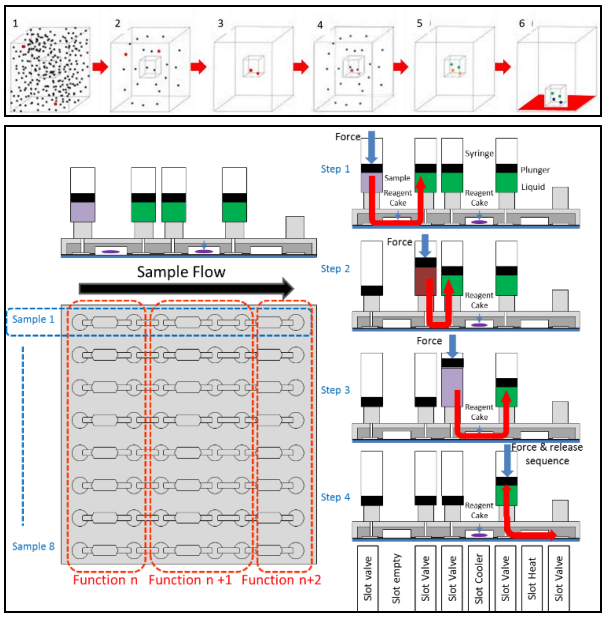

Figure 1. Schematic view of a possible configuration of a LOC during the different steps.

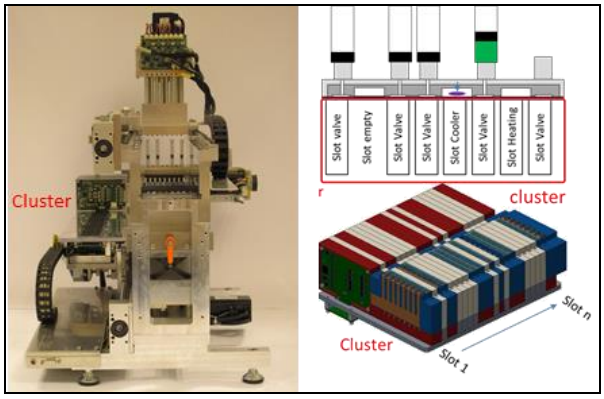

Figure 2. Left) Picture of a Control Unit. Centre) Schematic representation of a LOC laying on top a cluster. Right) Designs of the different slots.

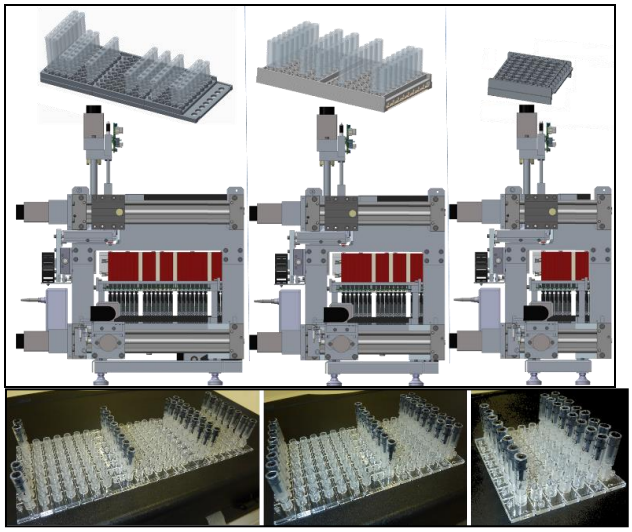

Figure 3. Different designs of the IVD system (top) depending on the number of Chip Units (bottom). Pictures from Left to right) three-Two and One Chip Unit. In red are pictured the slots.

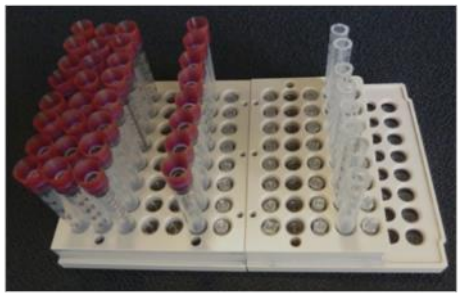

Figure 4. View of a LabonaChip for NIPD, based on two chips assembled.

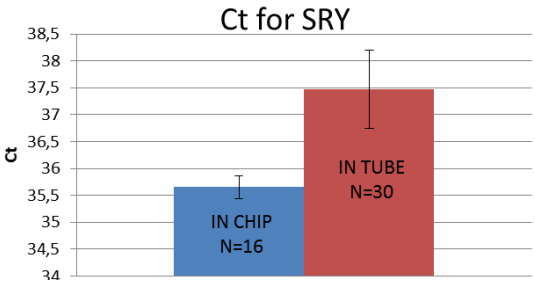

Figure 5:Ct amplification of 16 fetal DNA extractions on LOC with pregnant plasma carrying a male fetus (Ct of 35,5 versus 37,5).

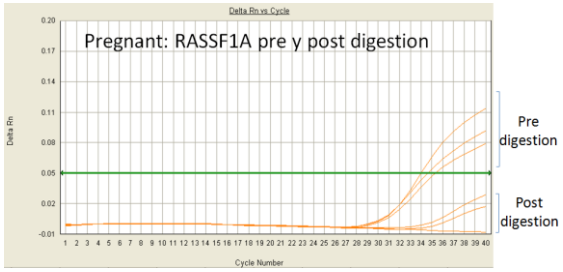

Figure 6. qPCR results targeting RASSF1A obtained with tfDNA digested in chip from 25µl of extracted DNA from pregnant plasma.

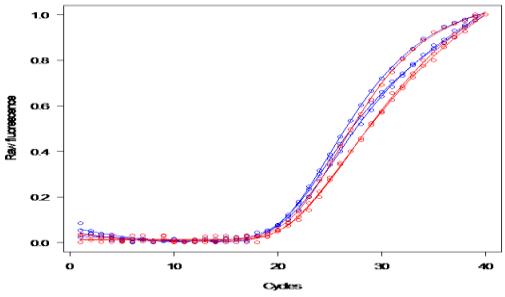

Figure 7. qPCR curves obtained in chip and in tube. The Ct values are very similar in chip and in tube.

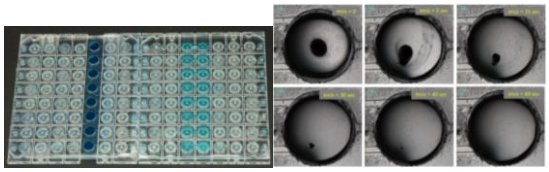

Figure 8. left) Device for MeDIP (right) Optical images showing the incubation sequence.

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