A Novel Lab-on-Disk System for Pathogen Nucleic Acids Analysis in Infectious Diseases

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Abstract The miniaturization of Real Time PCR (qPCR) systems is a crucial point towards the development of "genetic point-of-care" (PoC) that are able to offer sample-in-answer-out diagnostic analysis. Centralized laboratories and specialized staffs are needed for conventional DNA analysis. To solve this issue, we propose an innovative easy-to-use PoC technology based on a Lab-on-Disk miniaturized system, integrating nucleic acids extraction process based on Mags-Beads technology and detection based on qPCR. Lab-on-Disk system is composed by a polycarbonate disk with reagent-on-board for DNA extraction and a qPCR silicon-chip. A customized reader integrating electronic and optical modules was developed for driving the polycarbonate disk. Here we present results in the detection of Hepatitis B Virus (HBV) genome.

Keywords Pathogen DNA extraction/detection · Microfluidics · Magnetic beads QPCR

1 Introduction

The development of diagnostic technologies able to perform complete DNA analysis in a miniaturized-integrated-automated way is one of the fascinating fields in biomedical research. The scientific innovation is moving towards the decentralization of molecular diagnostics from the hospital's core laboratory. The aim is to allow massive diagnostic screening and better facing the threat of genetic and infectious diseases. Its clinical utility could be much more relevant, for example, in developing

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countries where infectious disease diagnosis is still a challenge due to poor clinical laboratory infrastructures and cost constraints [1].

Based on the above considerations, multidisciplinary research teams have spent significant efforts to develop innovative technologies and new chemical strategies for DNA analysis, leading to the introduction of the "genetic Point-of-Care" (PoC) [2, 3]. These systems must integrate and automate all steps necessary for the molecular diagnostics, including the DNA sample preparation (extraction and purification) and detection (identification and quantification).

The DNA preparation from biological samples represent one of the major issues in genetic PoC approach, since several starting materials can be employed (blood, urine, saliva etc.) and, consequently, complex architectures and protocols are needed.

Strategies for DNA extraction evolved from liquid–liquid purification method, such as isolation by precipitation with phenol-chloroform [4, 5], to most advanced liquid–solid purification systems, mostly, based on silica in the form of micro-filter mounted in a plastic column or layers covering magnetic beads [6, 7].

However, despite the improvements in terms of yield quality and protocol safety, DNA purification methods still have many widespread limitations.

One of those is the volume and the amount of biological sample required to trigger all procedure. From liquid-liquid to solid-phase extraction, in fact, hundreds of microliters to milliliters volumes are used; this implies invasive biological samplings from patients for diagnosis.

Another common drawback is the miniaturization and integration of the purification technology in a single portable device. A lot of stuff is required to perform the whole experiment of genetic material isolation, frequently with a complex architecture to manage the fluidic steps, which implies an increase of the design complexity, high costs and a laboratory with dedicated and specialized staff.

A solution to all the drawbacks, above reported, is brought by the microfluidic technology [8]. Microfluidic platforms are extremely attractive thanks to the number of advantages, they present, compared to macroscopic equivalents. The small volumes, required for the experimental setup, reduce sample and reagent consumption. Moreover, reduced size of device implies the possibility of miniaturization and integration, so the possibility of working outside the laboratory.

The literature reports few examples of miniaturized sample preparation devices; many of these are developed using plastic materials and include a complex microfluidic network that manages fluid movement, mixing, splitting etc. [9–17]. Plastic materials for integrated microfluidics have the advantage of low cost but the miniaturization, integration and automation required by some of the extraction steps (i.e. lysis) remain the main limitations towards the development of a point-of-care device.

Another issue in PoC is the detection of genetic material.

The DNA analysis is, commonly, based on the Real Time PCR (qPCR) method. In literature, various PCR based PoC have been reported for DNA detection of different pathogen microorganisms. Fernandez-Carballo et al. in 2016 presented a portable and low-cost point-of-care (PoC) system based on continuous flow PCR for quantitative detection of Chlamydia trachomatis and Escherichia coli [18]. Hsien et al. described a sequence-specific electrochemical DNA technology (E-DNA) able

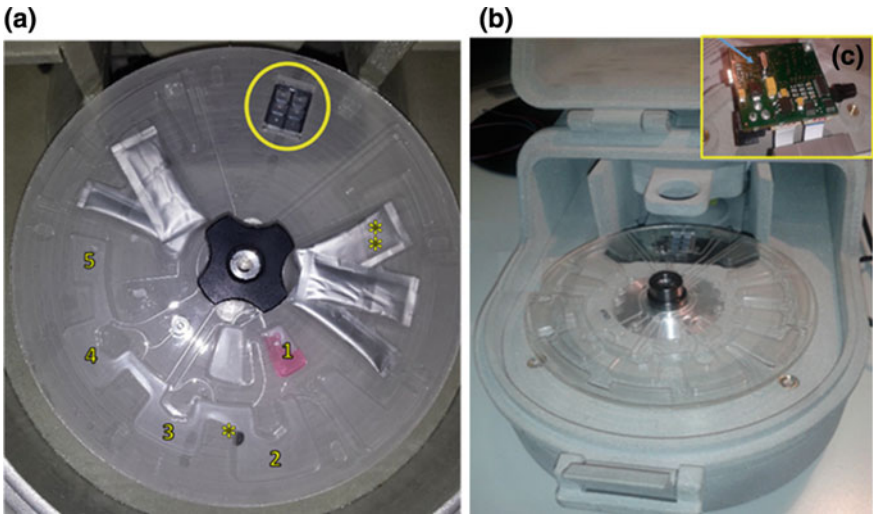

Fig. 1 **a** Lab-on-Disk: sample (1), lysis (2), binding (3), washing (4) and elution (5) chamber; magnetic beads (*); buffer stickpacks (⁑); qPCR chip (yellow ring). **b** Disk-reader. **c** Detail of electronic board for qPCR detection

to detect up to 100 copies per ml of S. typhimurium by loop-mediated isothermal amplification, 10 TCID50 for H1N1 influenza virus, and 300 copies (in 50 μL) of Salmonella enterica [19]. Other research teams reported miniaturized devices based on PCR using innovative transduction methods for the detection of pathogen species such as: Escherichia coli (by using piezoelectric-excited cantilever sensors) [20]; Neisseria meningitidis and HBV (using electrochemical transduction) [21–23]; Staphylococcus aureus (by means of graphene oxide based fluorescent probes) [24]; Mycobacterium tuberculosis (using surface plasmon resonance) [25]. Example of genetic SNP (Single Nucleotide Polymorphisms) application are, also, reported [26].

However, among all systems, reported, the miniaturization of the qPCR amplification system, together with the possibility of a sample-in answer-out diagnostic analysis, enabling shorter analysis times, reducing reagent consumption, minimizing risk of sample contamination and enhancing the assay performance (such as sensitivity, specificity and limit of detection), are common drawbacks still to be solved [27].

In this sense, we introduced an innovative Lab-on-Disk microfluidic technology, shown in Fig. 1a, for the complete analysis of pathogen DNA in infectious diseases.

A polycarbonate module, containing the DNA extraction reagent-on-board for the Magnetic-Beads based purification, and a silicon chip, for the qPCR detection of extracted DNA, compose the disk. This is introduced, then, into a specific disk-reader (Fig. 1b) to perform the rotations and amplification protocols.

The disk is able to perform sensitive and high throughput analysis of Hepatitis B Virus genome from a biological sample, overpassing all limitations towards the genetic PoC approach.

2 Materials and Methods

2.1 Chemicals and Reagents

Stock solutions of Hepatitis B virus (HBV) clone complete genome (ref product 05960116), consisting of the HBV genome 3.2 kbps and a plasmid PBR322 vector 3.8 kbps in TE (Tris 10 mM, EDTA 1 mM, pH = 8), and the HBV real time PCR kit (ref product FO2 HBV MMIX KIT 48) were purchased from CLONIT and used according to the Instructions for Use.

2.2 Extraction Experiments

To test the extraction efficiency of polycarbonate module, 10^6 copies/μl of HBV clone were dissolved in 200 μl of Milli-Q water.

Once loaded the sample, a series of rotations allowed the beads to move through all reaction chambers (Fig. 1a), so that the HBV DNA can be adsorbed and purified. To quantify the eluted DNA, a qPCR amplification and cycles threshold (Ct) analysis was performed using the Q3 platform, developed by STMicroelectronics [27, 28]. For the qPCR experiment, 5 μL of both starting and eluted DNA were pipetted into the qPCR chip (described in Sect. 2.3), and preloaded with 10 μL of the HBV kit master mix. The thermal protocol used for qPCR reaction is reported in Table 1. The same experimental conditions have been applied for Magazorb and Qiagen extraction yield analysis.

2.3 Real Time Amplification on the Chip

A miniaturized silicon chip, integrating temperature sensors and heaters for the qPCR amplification and detection of purified DNA (developed by STMicroelectronics [27, 28]), has been assembled to the disk platform.

The chip was thermally and optically driven by an electronic miniaturized board, inside the disk-reader (Fig. 1c). The fluorescence signals were collected by the CCD detector inside the board and analyzed by a smart detection software [27].

Table 1 Real time PCR thermal protocol

Step	Temperature (°C)	Time (s)
Initial denaturation	99	600
Denaturation	99	15
Annealing	62	60
Extension	50	1

For the qPCR detection test, a negative sample (1 μL of water + 14 μL of HBV master mix) and three positive controls (1 μL of 1×10^6, 1×10^5 and 1×10^3 cps μL^{-1} + 14 μL of HBV master mix) of HBV clone genome were loaded on chip. Thermal protocol was the same reported in Table 1.

The HBV samples were amplified, in parallel, by the commercial Applied Biosystems 7500 as comparison.

3 Results and Discussions

3.1 Module for DNA Extraction

Extraction data from HBV purification on Lab-on-Disk, compared to those from gold standard Magazorb and Qiagen kit, are reported in Table 2.

As shown, the disk platform is able to perform a complete purification process of HBV clone DNA, with an eluate of about 10^3 cps/μl (29.92 Ct).

The protocol for Lab-on-Disk extraction is extremely simplified, since no more than 1 step is required for the experiment (as described in table), with an elution yield comparable to the Magazorb and Qiagen one (the 2 Ct gap is due to a partial DNA retention on beads after the elution in Lab-on-Disk).

3.2 Module for DNA Detection

Results of DNA amplification and fluorescence imaging from qPCR on integrated chip in Lab-on-Disk are shown in Fig. 2a, b, respectively.

In order to estimate the detection efficiency, the qPCR data of 10^6-10^5-10^3 cps/μl HBV DNA amplification on chip have been compared to the gold standard Applied Biosystems 7500 and the Ct values, from quantification, are reported in Table 3.

These data show a detection efficiency improvement (about 1 Ct for all HBV samples) in the case of integrated silicon chip, respect to the commercial instrument. These enhancements in sensitivity and time, since less PCR cycles are required for

Table 2 Extraction data comparison

KIT	Protocol step	Sample volume (μl)	Elution buffer volume (μl)	Eluate volume (μl)	qPCR Ct
Prep disk	1	200	200	200 ± 5	29.92
Magazorb	15	200	200	200 ± 5	27.98
Qiagen	11	200	200	200 ± 5	27.91

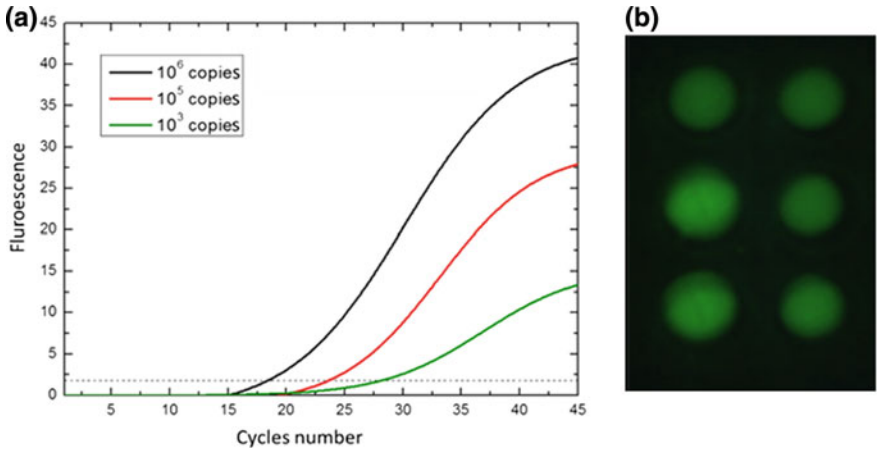

Fig. 2 **a** Lab-on-Disk amplification plot. **b** Fluorescent imaging of qPCR chip

Table 3 Detection data comparison

DNA (copies/μl)	Lab-on-disk (Ct)	Applied biosystems (Ct)
10^6	19.4 ± 0.2	19.9 ± 0.2
10^5	23.9 ± 0.3	23.9 ± 0.2
10^3	28.5 ± 0.5	29.9 ± 0.4

the DNA quantification, result from a perfect synergy between the specific chip architecture and the smart detection software used for the analysis.

4 Conclusions

In this work, we introduce a Lab-on-Disk system for the extraction and detection of Hepatitis B Virus genome. The system is composed by a reader and a polycarbonate disk integrating all reagents for Mag-Beads purification and a miniaturized silicon chip for qPCR detection.

DNA extraction test revealed that the system is able to perform a complete purification of HBV genome in one-step, since no actions of operator are required. In parallel, detection test on integrated qPCR chip in Lab-on-disk, showed that the system is also able to quantify HBV DNA with an improvement of sensitivity of about 1 Ct, respect to the commercial qPCR platforms, which means an optimization in time and material consuming.

These results, together with the miniaturization and integration of all stuff inside the portable disk-reader, are important features towards the identification of Lab-on-Disk as new Point-of-Care technology suitable for infectious disease diagnosis.

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