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ARTICLE

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An integrated biosensor platform for extraction and detection of nucleic acids

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Abstract

The development of portable systems for analysis of nucleic acids (NAs) is crucial for the evolution of biosensing in the context of future healthcare technologies. The integration of NA extraction, purification, and detection modules, properly actuated by microfluidics technologies, is a key point for the development of portable diagnostic systems. In this paper, we describe an integrated biosensor platform based on a silicon–plastic hybrid lab-on-disk technology capable of managing NA extraction, purification, and detection processes in an integrated format. The sample preparation process is performed by solid-phase extraction technology using magnetic beads on a plastic disk, while detection is done through quantitative real-time polymerase chain reaction (qRT-PCR) on a miniaturized silicon device. The movement of sample and reagents is actuated by a centrifugal force induced by a disk actuator instrument. The assessment of the NA extraction and detection performance has been carried out by using hepatitis B virus (HBV) DNA genome as a biological target. The quantification of the qRT-PCR chip in the hybrid disk showed an improvement in sensitivity with respect to the qRT-PCR commercial platforms, which means an optimization of time and cost. Limit of detection and limit of quantification values of about 8 cps/reaction and 26 cps/reaction, respectively, were found by using analytical samples (synthetic clone), while the results with real samples (serum with spiked HBV genome) indicate that the system performs as well as the standard methods.

KEYWORDS

biosensors, extraction, microfluidics, nucleic acids, qRT-PCR, silicon chip

1 | INTRODUCTION

The growing demand for innovative methodologies for the analysis of nucleic acids (NAs) has prompted research on the development of portable diagnostic systems (Callari, Petralia, Conoci, & Sortino, 2008; Sortino, Petralia, Condorelli, Conoci, & Condorelli, 2003). Miniaturization and integration of whole-NA analysis processes (extraction, amplification, and detection), together with easy-to-use systems, are key for the development of next-generation molecular platforms, the so-called genetic point-of-care (POC). They are suitable to be used in a decentralized environment and, therefore, can have a huge impact on developing countries characterized by poor clinical laboratory infrastructures (Petralia & Conoci, 2017; Petralia et al., 2013; Yager, Domingo, & Gerdes, 2008).

The integration of NA analysis in portable systems requires the integration of specific technological modules that manage the extraction and purification processes and the detection step.

Several examples of NA separation/purification technologies, integrated on miniaturized diagnostic systems, have been proposed in the literature. Among these, solid-phase extraction (SPE) is one of the most effective extraction method based on electrostatic interactions between NA molecules and SiO_2 surfaces (Berensmeier,

2006). It has been implemented in several formats, such as silicon pillars (Petralia, Sciuto, & Conoci, 2017), filter membranes (Mauk, Song, Liu, & Bau, 2018), and magnetic beads (MagaZorb DNA Mini-Prep Kit Technical Bulletin, 2011). When magnetic beads are used, the silica surface covers microparticles' magnetic core, making the NA–SiO_2 electrostatic interactions tunable by using proper washing and elution buffers to obtain purified and concentrated NA samples. Thanks to the technological evolution in the miniaturization of magnetic beads technology, the SPE method has become very appealing to be easily integrated on miniaturized diagnostic systems.

Presently, quantitative real-time polymerase chain reaction (qRT-PCR) amplification is used as the reference method for the detection of NAs, and it guarantees high sensitivity and good specificity. It exhibits limit of detection (LOD) values of up to 10 cps/reaction.

Genetic POC systems require the integration of NA extraction/purification module with the detection module. In this context, fluidic connections are a key factor. These can be actuated by various methodologies, such as pumping force (Quake & Scherer, 2000), electrowetting (Petralia, Motta, & Conoci, 2019), electrovalve (Haeberle & Zengerle, 2007; Thorsen, Maerkl, & Quake, 2002), or centrifugal force (Mark, Haeberle, Roth, von Stettenza, & Zengerlez, 2010) depending on the technology employed and the specimen complexity. Urine, blood, and sputum, for example, are complex specimens (in terms of density, composition, pH value, etc.), which require complicated microfluidic architectures for lysis and separation, together with elaborated biological and microfluidics protocols (Quail et al., 2012; Mabey, Peeling, Ustianowski, & Perkins, 2004).

In this scenario, the integration of silicon technologies such as micro-electromechanical systems and very large-scale integration, together with microfluidics, is very intriguing to achieve full integration of NA testing protocols on miniaturized and portable diagnostic devices (Fernández-Carballo et al., 2016; Guarnaccia et al., 2014; Hsieh et al., 2015; Tong et al., 2019). The main advantages of silicon are low heat capacity, good thermal conductivity, good biocompatibility, chemical derivatization, and the possibility to produce patterned structures, which increase the surface-area ratio, improving the efficiency of NA extraction and detection (Leonardi et al., 2018; Petralia et al., 2015; Valli et al., 2006). 3D printing technology and numerical tools are useful techniques to generate models for the simulation of surface to control the shape and size of pores as well as the distribution of the pore's network inside the sample (Zerhouni, Tarantino, Danas, & Hong, 2018).

In addition, the silicon technology allows effective and easy integration of plastic microfluidic modules and microelectronics circuitry that imprint the so-called "intelligence on-board," which is essential toward the integration process of multiple functional modules.

Among a huge series of microfluidic technologies developed so far (Chin, Linder, & Sia, 2012; Smith et al., 2016), one of the best performers is the lab-on-disk technology, in which the centrifugal fluidic actuation induces fluid movement, mixing of reagents, and separation of beads (Gilmore, Islam, & Martinez-Duarte, 2016).

Recently, Tong et al. introduced an example of the lab-on-disk microfluidic approach for genomic purification, consisting of a sample processing disk, a magnet module, and an external instrument for process management (Petralia et al., 2017).

In this study, we propose an innovative integrated biosensor system (lab-on-disk platform), based on a hybrid silicon–plastic disk system that can perform in the POC format the extraction, purification, and detection of a few copies of an analytical sample consisting the hepatitis B virus (HBV) genome. This is achieved by integrating magnetic beads SPE extraction method and qRT-PCR detection, combining centrifugal microfluidics and silicon technology at different levels to achieve complete integration and automation of the NA analytical process. Both the microfluidic layout and centrifugal forces applied to the hybrid disk allow the mixing of the extraction reagents with the starting biological sample and the flowing of the purified NAs for the subsequent detection area. A miniaturized silicon qRT-PCR chip is assembled on the detection area of the disk for NA amplification.

The assessment of the NA extraction and detection performances of the lab-on-disk platform by using an HBV biological target are presented. In particular, the analytical performances in terms of LOD and limit of quantification (LOQ) using a synthetic clone are first discussed. Then, the assessment of the platform with mock samples (simulating real samples) is finally described and commented upon.

2 | EXPERIMENTAL DETAILS

2.1 | Chemicals and reagents

Stock solutions of HBV clone complete genome (ref product 05960116), consisting of the HBV genome 3.2 kbps and a plasmid PBR322 vector 3.8 kb 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. In detail, 10 μl of a Clonit Master Mix solution containing PCR buffer (1×), Taq DNA polymerase, 0.5 μM of each forward sequence (GGTGAGTGATTGGAGGTT) and reverse sequence (CAACATCAGGATTCCTAG) primers and 2 μl of the standard HBV clone were used.

The mycobacterium tuberculosis clone (MTB) was purchased from Clonit (ref product RT-19, "Detection of the Mycobacterium complex with Real Time PCR") and used as received.

Mock samples from human serum were prepared by adding a volume of 20 μl of a stock sample of HBV synthetic clone (1×10^5 cps/μl) in a volume of 180 μl of human serum sample to obtain a final concentration of HBV clone 10^4 cps/μl.

2.2 | Lab-on-disk platform

The lab-on-disk platform is composed of (a) a disposable disk (hybrid disk) for NA extraction, amplification, and detection and (b) a lab-on-disk reader for the management of microfluidics, thermal, and optical protocols.

2.2.1 | Hybrid disk

The hybrid disk is a hybrid structure composed of a plastic microfluidic disk for NA collection and purification (prep disk), and a silicon qRT-PCR chip for the NA amplification and detection (Figure 1).

In detail, the prep disk (13 cm in diameter, 2 mm in thickness) was developed in cooperation with HSG-IMIT (Germany). It is fabricated in polymethyl methacrylate foils combining laser cutting and hot embossing technologies and was properly designed for microfluidic actuation by centrifugal forces (Figure 1b). The silicon chip for qRT-PCR (Figure 1a–c) was developed by STMicroelectronics according to the process steps described in a previous work (Guarnaccia, Iemmolo, Petralia, Conoci, & Cavallaro, 2017).

The device is composed of a silicon chip of 1.7× 1.3 cm (bottom part) assembled with a polycarbonate ring (top part) containing six reaction chambers of 20 μl each (Figure 1c). The bottom part of the silicon chip integrates the temperature sensors and heaters, reaching a temperature control accuracy of ±0.2°C, a heating rate of 15°C/s, a cooling rate of 8°C/s, and a temperature resolution of 0.1°C. The top polycarbonate ring is manufactured by molding technology. Each top microreactor is composed of cylinders with different diameters, 3.8 mm for the top cylinder (volume of ~9.6 μl) and 3 mm for the bottom one (volume of ~14.1 μl). The two parts (silicon and polycarbonate ring) are glued and well aligned with each other (with a conductive resin DELO MONOPOX) to form six microreactors with a final total volume of 25 μl each. The device is capable of performing the amplification and detection of the collected NAs via RT-PCR.

The prep disk is glued onto a plastic holder of polycarbonate (disk of 13 cm in diameter, 0.5 cm in thickness) mounting the silicon qRT-PCR chip for the detection module (Figure 1d).

2.2.2 | Lab-on-disk reader

The disk reader consists of an instrument integrating all the mechanical, electronic, magnetic, and optical components to perform NA analysis on lab-on-disk (Figure S1a). In particular, it contains a holder to fix and manage disk rotations, necessary to perform the NA extraction, two magnets for beads collection (Figure S1b), and an electronic module for the thermal/optical control in the NA detection step. By tuning the

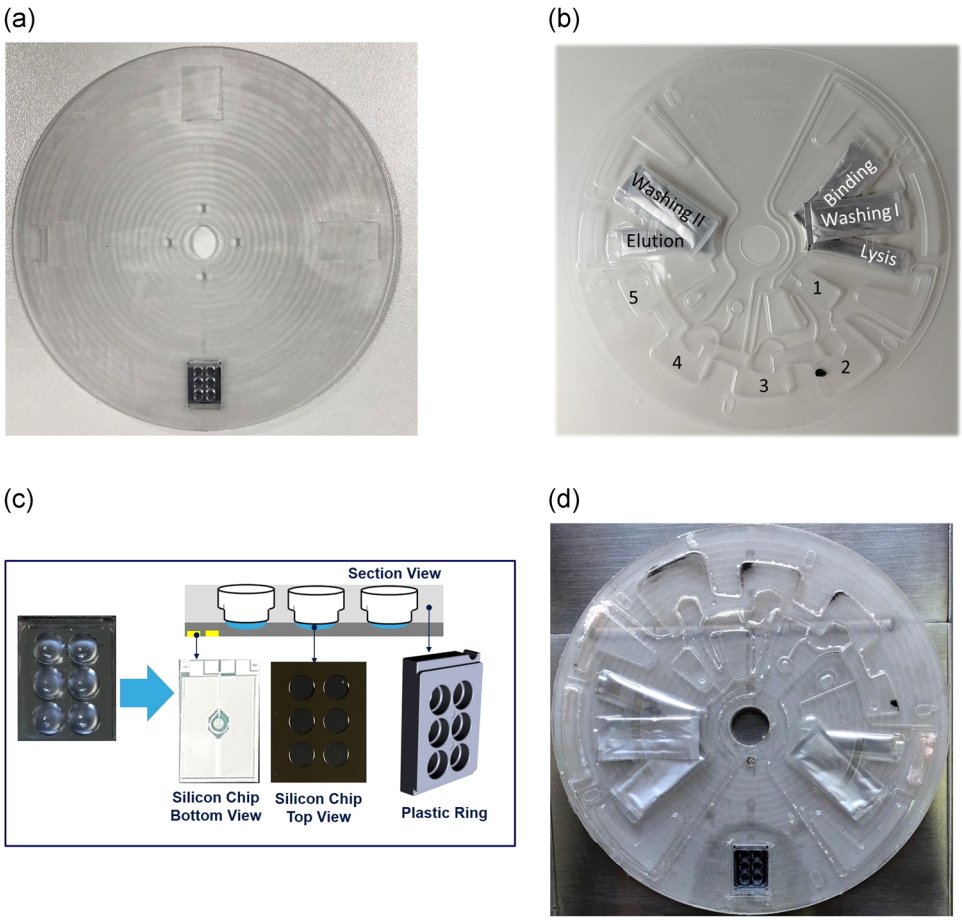

FIGURE 1 Hybrid disk: (a) polycarbonate disk with quantitative real-time polymerase chain reaction (qRT-PCR) silicon chip; (b) prep disk composed of: (1) sample loading chamber, (2) lysis chamber with paramagnetic beads preloaded, (3) binding chamber, (4) washing chamber, (5) elution chamber, five reagent buffer stick packs for the process steps; (c) silicon chip: section view, temperature sensors and heaters, integrated microchambers plastic ring; details of qRT-PCR silicon chip and (d) lab-on-disk biosensor [Color figure can be viewed at wileyonlinelibrary.com]

rotation speed, the instrument allows the actuation of disk microfluidics performing various process steps, such as mixing of sample with reagents, transferring of sample between connected chambers, interaction and mixing with magnetic beads, and reagent stick pack opening.

The electronic module, placed behind the reader lid, was designed to manage the thermal and optical modules.

The optical module consists of two independent optical channels: for multiple fluorescent reporters (FAM, VIC®), the light excitation is provided by a light-emitting diode (LED) source, (emission range 470 –530 nm) and a charged-couple device (CCD) optical detector to collect the fluorescent signal during NA amplification.

The thermal module is composed of a board and connectors to drive the temperature sensor and heater integrated on a silicon chip. Finally, a software package completes the instrument for the management of the PCR process and data analysis is carried out. The fluorescence signals collected by CCD are analyzed by a smart detection software (Spata, Castagna, & Conoci, 2015).

The NA extraction and purification processes are actuated to the disk by centrifugal forces managed by the reader. Table 1 reports the protocol steps used for this process.

The total process time is about 15 min for extraction and purification and about 60 min for qRT-PCR detection and data analysis.

2.3 | NA extractions and detection experiments

The NA extraction and detection experiments were performed using a volume of 200 µl of an analytic sample containing 1×10^6, 1×10^5, 1×10^4, and 1×10^3 copies/reaction of HBV clone and a volume of 200 µl of a mock sample, which was prepared by human serum spiking HBV clone to a final concentration of 10^4 cps.

The NA detection experiments were performed directly on a silicon chip (with reagent on board). In detail, a volume of about 5 µl of extracted solution was moved from the elution chamber to the silicon chip chamber, containing all primers and reagents for the amplification reaction and 6 µl of wax to avoid evaporation of samples during the thermal cycles. Finally, the hybrid disk was inserted into the optical module and amplification was carried out with the following thermal protocol: initial denaturation step (10 min at 95°C), followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Negative samples were prepared by substituting the HBV clone with the same amount of water. Each experiment was replicated three times. The optical parameters of the disk reader setting were as follows: gain: ×4.00; LED power: 7; frame rate: 0.5 for the FAM channel.

For comparison, the same experimental conditions were used for HBV sample amplification by the commercial Applied Biosystems 7500.

3 | RESULTS AND DISCUSSION

3.1 | Lab-on-disk platform: hybrid disk

Figure 1 schematically depicts the hybrid disk of the lab-on-disk platform. It consists of a polycarbonate disk (Figure 1a) integrating a plastic microfluidic module for NA extraction and purification (prep disk, Figure 1b) and a miniaturized silicon chip (Figure 1c) for NA amplification and detection via qRT-PCR. The final hybrid disk is reported in Figure 1d.

Proper values of speed and acceleration for specific times induce microfluidics actuation on the disk that moves the sample through the reaction chambers from 1 to 5, allowing the reagent stick pack to open and the solution to mix. Figure 2 illustrates the whole fluidic process and the parts of lab-on-disk involved. A volume of 200 µl of biological sample is loaded on chamber 1 (*loading step*), and a starting process (52 s) initializes the cycle, moving the sample and the lysis reagents (from lysis stick pack) into chamber 2 (*lysis step*), where magnetic beads (Magazorb DNA Mini kit, Promega) are preloaded. After the lysis process (29 s), the sample and the beads are mixed in the same chamber for the next step, together with a specific buffer released from the stick pack (binding) containing 1.25 M sodium chloride and 10% polyethylene glycol, to induce the NA–beads interaction (*binding step*). Thereafter, the sample is moved, together with the washing buffer from stick pack washing I and stick pack washing II into chambers 3 and 4 (washing steps). Finally, in the last chamber 5, a specific buffer from stick pack elution induces the removal of cation bridges to separate the NA from the bead surface for final collection (*elution step*). A magnet (diameter ~5 mm), integrated in the reader, induces the separation of the sample from the beads (Figure 3b, inset). Five microliters of the collected sample is then transferred to each silicon chip chamber to perform the qRT-PCR detection phase in the optical detection module (*detection step*). The details of all the microfluidic protocols above described are presented in Table S2.

The assessment of the NA extraction and detection performances of the lab-on-disk platform has been carried out by using HBV DNA genome as a biological target. The following sections describe both the analytical evaluation carried out using a synthetic clone of both the prep disk (for extraction capabilities) and the fully integrated hybrid disk (for the total performances in the NA analysis [extraction and detection]) and the assessment with mock samples (simulating real samples) to evaluate the platform performance in the real case.

TABLE 1 NA analysis process steps

Process	Main step	Disk portion	Time (s)
NA extraction and purification	Lysis	2	29
	Binding step	3	149
	Wash steps	4	299
	Elution buffer	5	94
	Transfer		88
NA detection	qRT-PCR detection	Silicon chip	3,600
	Data analysis and result	–	300

Abbreviation: NA, nucleic acid.

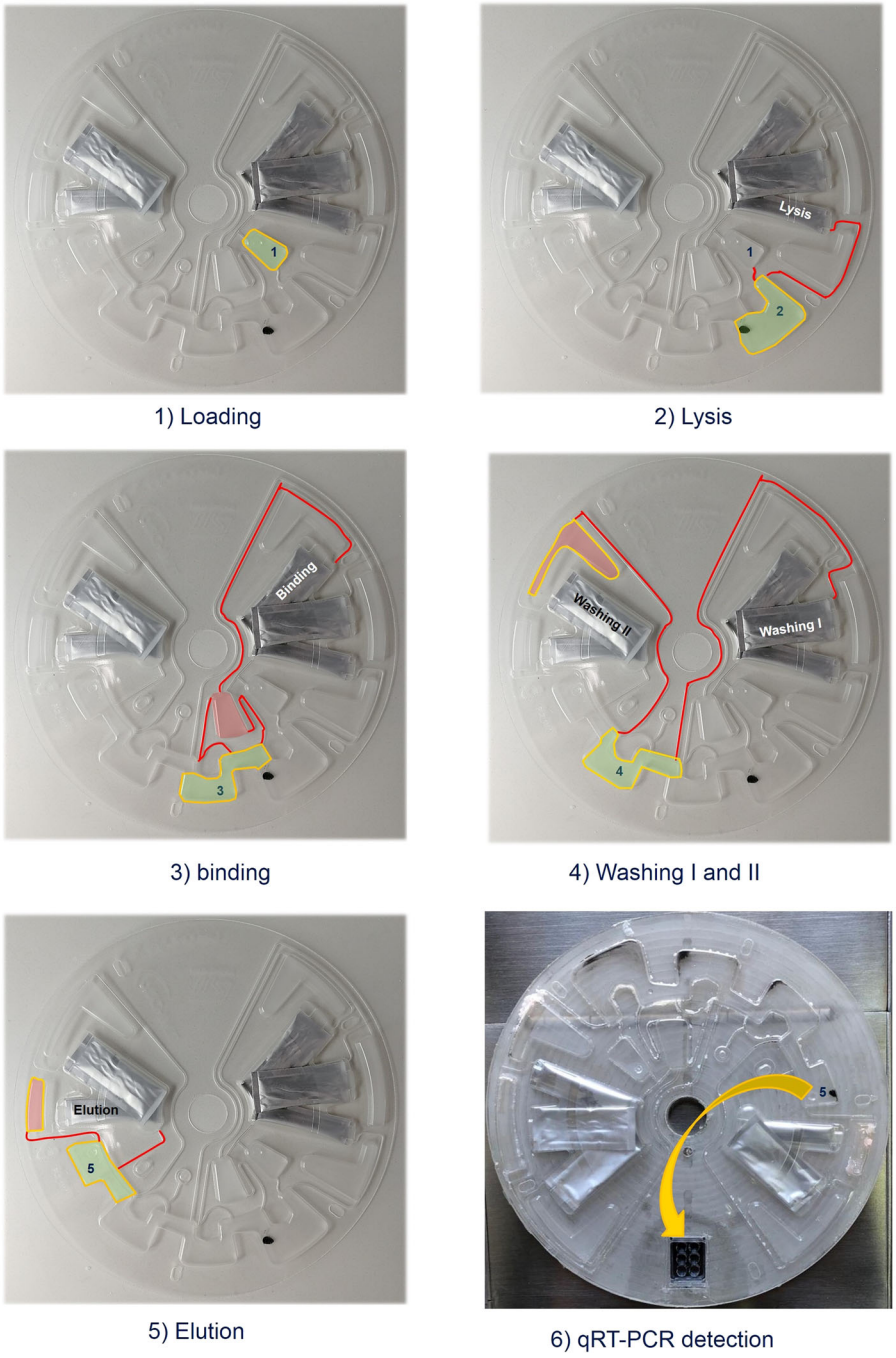

FIGURE 2 Lab-on-disk parts involved in nucleic acid analysis. Main process steps: loading, lysis, binding, washing, elution, and detection. qRT-PCR, quantitative real-time polymerase chain reaction [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Prep disk analytical assessment

The first part of the study was devoted to assess the performance of the extraction module with analytical samples. This was evaluated by loading aliquots (200 μl) containing different amounts (1×10^3, 1×10^4, 1×10^5, and 1×10^6 cps/reaction) of the HBV clone sample. After the extraction and purification phase, the amount of HBV copies on extracted sample was quantified by qRT-PCR on standard equipment. For comparison, the whole extraction process was performed using a standard Qiagen extraction kit. Table 2 illustrates the results of qRT-PCR (C_t value) for all extracted samples by a prep disk module and by a standard Qiagen platform.

It can be noticed that the hybrid disk extraction efficiency is comparable in terms of C_t value and standard deviation, with those of the commercial kit. This highlights that the prep disk extraction technology exhibits the same efficiency as the reference gold standard currently used for NA extraction.

3.3 | Functional tests of hybrid disk

Functional tests of both extraction and detection modules of hybrid disk were performed using 1×10^3, 1×10^4, 1×10^5, 1×10^6 copies/μl of the HBV genome sample. The samples were loaded onto the

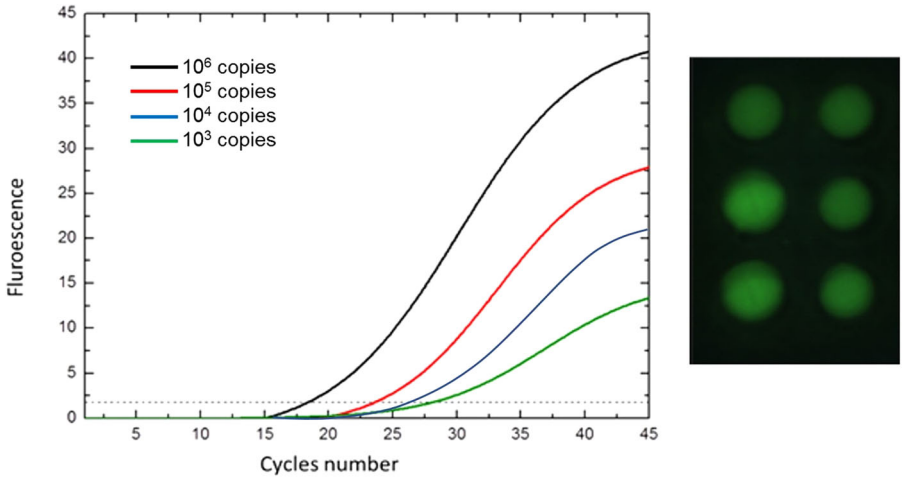

FIGURE 3 On-board quantitative real-time polymerase chain reaction output: (a) fluorescence amplification plot for 1×10^3 (green line), 1×10^4 (blue line), 1×10^5 (red line), and 1×10^6 (black line) copies per microliters of hepatitis B virus genome, collected from on-board extraction; (b) fluorescence image of silicon chip after the amplification cycles [Color figure can be viewed at wileyonlinelibrary.com]

hybrid disk and once eluted from the microfluidic magnetic beads, they were fluidically transferred to the wells of the qRT-PCR silicon chip for final quantification. The results of HBV NA amplification and fluorescence imaging are shown in Figure 3a,b, respectively. Detection efficiency was evaluated by comparing qRT-PCR data from the hybrid disk detection module with those of the Applied Biosystems 7500 gold standard; the C_t values, from the quantification, are reported in Table 3.

The data show that the integrated extraction-detection capability of the hybrid disk enhances the performance quantification of about 1 C_t, with respect to the commercial instrument, thanks to the perfect synergy between the properties and the layout of the miniaturized silicon chip, the software and electronic management integrated inside the reader and is used for the analysis.

3.4 | Lab-on-chip platform specificity, LOD, and LOQ

To investigate the specificity of the lab-on-disk system, three samples consisting of (a) HBV (1×10^4 cps) and MTB clone (1×10^4 cps), (b) MTB (1×10^4 cps), and (c) HBV (1×10^4 cps) were tested. The extraction and qRT-PCR detection resulted in negligible amplification (C_t value: 39.0) for the sample containing MTB, and comparable C_t values for the samples containing HBV + MTB (26.4) and alone HBV (26.0), suggesting that lab-on-disk system retains the qRT-PCR specificity.

TABLE 2 Prep disk analytical assessment

HBV sample (copies)	Prep disk (C_t)	Qiagen platform (C_t)
10^6	20.4 ± 0.2	20.0 ± 0.1
10^5	24.9 ± 0.1	24.0 ± 0.1
10^4	26.1 ± 0.2	25.4 ± 0.1
10^3	29.9 ± 0.3	28.9 ± 0.2

Abbreviation: HBV, hepatitis B virus.

To characterize both the LOD and the LOQ of lab-on-chip platform, we used the standard Equations (1) and (2), plotting the C_t values on the measured amount of the HBV clone (Shabir, 2003).

$$\text{LOD} = \frac{\text{standard error}}{\text{slope}} \times 3.3, \quad (1)$$

$$\text{LOQ} = \frac{\text{standard error}}{\text{slope}} \times 10. \quad (2)$$

Figure 4 reports the results obtained in this study. A linear relationship between C_t and the amount of HBV cps was found. As only the first data point was in a linear regime, we plotted the standard calibration line using the first three points, obtaining the following equation: $Y = -2.82x + 37.6$, where the slope (2.93 log cps/μl) indicates the sensitivity of the system. The standard error was estimated at 1.17. From Equations (1) and (2), we found an LOD value of about 8 cps and an LOQ value of 26.0 cps.

3.5 | Biological sample analysis with hybrid disk

To investigate the performance of our system assay in real cases, mock samples from human serum were prepared and tested with a hybrid disk system. Specifically, the HBV synthetic clone was added to the human serum sample at the final concentration of 1×10^4 cps. Then a volume of 200 μl of the mock sample was loaded onto a hybrid disk and analyzed. After the extraction process, the detection by qRT-PCR

TABLE 3 Comparison of qRT-PCR data from hybrid disk and conventional platform (Applied Biosystems 7500)

HBV sample (copies/μl)	Hybrid disk (C_t)	Conventional platform (C_t)
1×10^6	19.1	19.9
1×10^5	23.4	23.9
1×10^3	28.5	29.9

Abbreviations: HBV, hepatitis B virus; qRT-PCR, quantitative real-time polymerase chain reaction.

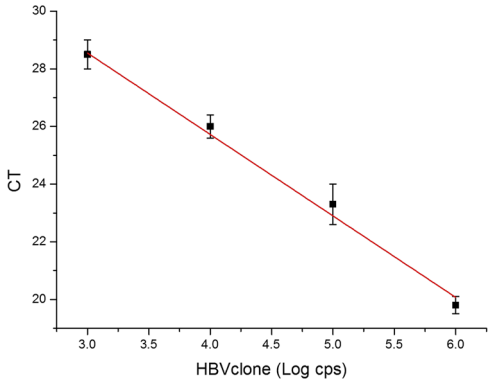

FIGURE 4 Limit of detection (LOD) calculation: qRT-PCR detection for different amounts of hepatitis B virus (HBV) clone (1×10^3, 1×10^5, and 1×10^6 cps). The C_t values were plotted against HBV clone amount on a logscale, the LOD was found to be 8.0 cps [Color figure can be viewed at wileyonlinelibrary.com]

module was obtained at a final C_t value of about 27.5 ± 0.2. The positive control was tested by performing the extraction process of a mock sample with Qiagen platform and the qRT-PCR detection experiments with Applied Biosystems equipment. The obtained results show a C_t value of about 27.0 ± 0.2 for the positive control. The data indicate that with the real specimen, the hybrid disk performs as well as the standard methods, although a slight decrease in efficiency has been found with respect to the data obtained for analytical samples (Table 2) both for the hybrid disk and for the standard platforms.

To confirm the presence of the HBV analytical target, the melting curves of the above-amplified products were collected and analyzed with proper analytical procedures. The results show a melting temperature of 61°C for the extracted sample according to the designed amplicon length.

These improvements also allow to save time and cost of the analytical approach, as fewer qRT-PCR cycles, genomic material, and reagents are needed to achieve the right sensitivity, which makes the quantification faster and cheaper.

4 | CONCLUSIONS

A biosensor platform based on a lab-on-disk system for the integrated extraction, purification, and detection of the pathogen genome (HBV) has been described. The core of the platform is a hybrid disk biosensor device integrating a plastic microfluidic disk for NA collection and purification and a silicon miniaturized chip for NA amplification and detection through qRT-PCR. Characterization and functional tests highlight that the hybrid disk performs an exhaustive one-step HBV genome purification, together with a high-performance qRT-PCR analysis. The quantification of integrated qRT-PCR chip in the hybrid disk showed an improvement in sensitivity with respect to the qRT-PCR commercial platforms, which means an optimization of time and cost. LOD and LOQ values of about 8 cps/reaction and 26 cps/reaction, respectively, were found by using analytical samples (synthetic clone), while the findings with real samples (serum with spiked HBV genome) indicate that the system performs as well as the standard methods.

The results presented herein are consistent with the idea of a portable and easy-to-use diagnostic technology paving the way for future development of genetic POC for the diagnosis of infectious diseases.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

Berensmeier, S. (2006). Magnetic particles for the separation and purification of nucleic acids. *Applied Microbiology and Biotechnology*, *3*, 495–504. https://doi.org/10.1007/s00253-006-0675-0

Callari, F. L., Petralia, S., Conoci, S., & Sortino, S. (2008). Light-triggered DNA release by dynamic monolayer films. *New Journal of Chemistry*, *32*, 1899–1903. https://doi.org/10.1039/B808118B

Chin, C., Linder, V., & Sia, S. (2012). Commercialization of microfluidic point-of-care diagnostic devices. *Lab on a Chip*, *12*, 2118–2134. https://doi.org/10.1039/c2lc21204h

Fernández-Carballo, B. L., McGuiness, I., McBeth, C., Kalashnikov, M., Borrós, S., Sharon, A., & Sauer-Budge, A. F. (2016). Low-cost, real-time, continuous flow PCR system for pathogen detection. *Biomedical Microdevices*, *18*, 34–40. https://doi.org/10.1007/s10544-016-0060-4

Guarnaccia, M., Gentile, G., Alessi, E., Scheider, C., Petralia, S., & Cavallaro, S. (2014). Is this the real time for genomics? *Genomics*, *103*, 177–182. https://doi.org/10.1016/j.ygeno.2014.02.003

Gilmore, J., Islam, M., & Martinez-Duarte, R. (2016). Challenges in the use of compact disc-based centrifugal microfluidics for healthcare diagnostics at the extreme point of care. *Micromachines*, *7*, 52. https://doi.org/10.3390/mi7040052

Guarnaccia, M., Iemmolo, R., Petralia, S., Conoci, S., & Cavallaro, S. (2017). Miniaturized real-time PCR on a Q3 system for rapid KRAS genotyping. *Sensors*, *17*, 831. https://doi.org/10.3390/s17040831

Hsieh, K., Ferguson, B. S., Eisenstein, M., Plaxco, K. W., & Soh, H. T. (2015). Integrated electrochemical microsystems for genetic detection of pathogens at the point of care. *Accounts of Chemical Research*, *48*, 911–920. https://doi.org/10.1021/ar500456w

Haeberle, S., & Zengerle, R. (2007). Microfluidic platforms for lab-on-a-chip applications. *Lab on a Chip*, *7*, 1094–1110. https://doi.org/10.1039/B706364B

Leonardi, A. A., Lo Faro, M. J., Petralia, S., Fazio, B., Musumeci, P., Conoci, S., … Priolo, F. (2018). Ultrasensitive label- and PCR-free genome detection based on cooperative hybridization of silicon

nanowires optical biosensors. *ACS Sensors*, *3*, 1690–1697. https://doi.org/10.1021/acssensors.8b00422

Mauk, M. G., Song, J., Liu, C., & Bau, H. H. (2018). Simple approaches to minimally-instrumented, microfluidic-based point-of-care nucleic acid amplification tests. *Biosensors*, *8*, 1–30. https://doi.org/10.3390/bios8010017

MagaZorb DNA Mini-Prep Kit Technical Bulletin. Instructions for Use of Product(s), MB1004; 2011; https://www.promega.com

Mark, D., Haeberle, S., Roth, G., von Stettenza, F., & Zengerlez, R. (2010). Microfluidic lab-on-a-chip platforms: Requirements, characteristics and applications. *Chemical Society Reviews*, *39*, 1153–1182. https://doi.org/10.1039/b820557b. and reference therein.

Mabey, D., Peeling, R. W., Ustianowski, A., & Perkins, M. D. (2004). Diagnostics for the developing world. *Nature Reviews Microbiology*, *2*, 231–240. https://doi.org/10.1038/nrmicro841

Petralia, S., & Conoci, S. (2017). PCR technologies for point of care testing: Progress and perspectives. *ACS Sensors*, *2*, 876–891. https://doi.org/10.1021/acssensors.7b00299

Petralia, S., Verardo, R., Klaric, E., Cavallaro, S., Alessi, E., & Schneider, C. (2013). In-Check system: A highly integrated silicon Lab-on-Chip for sample preparation, PCR amplification and microarray detection of nucleic acids directly from biological samples. *Sensors and Actuators B: Chemical*, *187*, 99–105. https://doi.org/10.1016/j.snb.2012.09.068

Petralia, S., Sciuto, E. L., & Conoci, S. (2017). A novel miniaturized biofilter based on silicon micropillars for nucleic acid extraction. *Analyst*, *142*, 140–146. https://doi.org/10.1039/C6AN02049F

Petralia, S., Motta, D., & Conoci, S. (2019). EWOD silicon biosensor for multiple nucleic acids analysis. *Biotechnology and Bioengineering*, *116*, 2087–2094. https://doi.org/10.1002/bit.26987

Petralia, S., Castagna, M. E., Cappello, E., Puntoriero, F., Trovato, E., Gagliano, A., & Conoci, S. (2015). A miniaturized silicon based device for nucleic acids electrochemical detection. *Sensing and Bio-Sensing Research*, *6*, 90–94. https://doi.org/10.1016/j.sbsr.2015.09.006

Petralia, S., Sciuto, E. L., Di Pietro, M. L., Zimbone, M., Grimaldi, M. G., & Conoci, S. (2017). Innovative chemical strategy for PCR-free genetic detection of pathogens by an integrated electrochemical biosensor. *Analyst*, *142*, 2090–2293. https://doi.org/10.1039/c7an00202e

Quake, S. R., & Scherer, A. (2000). From micro- to nanofabrication with soft materials. *Science*, *290*, 1536–1540. https://doi.org/10.1126/science.290.5496.1536

Quail, M., Smith, M. E., Coupland, P., Otto, T. D., Harris, S. R., Connor, T. R., … Gu, Y. (2012). A tale of three next generation sequencing platforms: Comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics*, *13*, 341. https://doi.org/10.1186/1471-2164-13-341

Sortino, S., Petralia, S., Condorelli, G. G., Conoci, S., & Condorelli, G. (2003). A novel photoactive self-assembled monolayer for immobilization and cleavage of DNA. *Langmuir*, *19*, 536–539. https://doi.org/10.1039/C0JM04359A

Smith, S., Mager, D., Perebikovsky, A., Shamloo, E., Kinahan, D., Mishra, R., … Korvink, J. G. (2016). CD-based microfluidics for primary care in extreme point-of-care settings. *Micromachines*, *7*, 22. https://doi.org/10.3390/mi7020022

Spata, M. O., Castagna, M. E., & Conoci, S. (2015). Image data analysis in qPCR: A method for smart analysis of DNA amplification. *Sensing and Bio-Sensing Research*, *6*, 79–84. https://doi.org/10.1016/j.sbsr.2015.10.006

Shabir, G. A. (2003). Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of Chromatography*, *987*, 57–66. https://doi.org/10.1016/S0021-9673(02)01536-4

Thorsen, T., Maerkl, S. J., & Quake, S. R. (2002). Microfluidic large-scale integration. *Science*, *298*, 580–584. https://doi.org/10.1126/science.1076996

Tong, R., Zhang, L., Hu, C., Chen, X., Song, Q., Lou, K., … Wen, W. (2019). An automated and miniaturized rotating-disk device for rapid nucleic acid extraction. *Micromachines*, *10*, 204. https://doi.org/10.3390/mi10030204

Valli, L., Casilli, S., Giotta, L., Pignataro, B., Conoci, S., Borovkov, V. V., … Sortino, S. (2006). Ethane-bridged zinc porphyrin dimers in Langmuir-Shäfer thin films: Structural and spectroscopic properties. *Journal of Physical Chemistry B*, *110*(10), 4691–4698. https://doi.org/10.1021/jp054974v

Yager, P., Domingo, G. J., & Gerdes, J. (2008). Point-of-care diagnostics for global health. *Annual Review of Biomedical Engineering*, *10*, 107–144. https://doi.org/10.1146/annurev.bioeng.10.061807.160524

Zerhouni, O., Tarantino, M.-G., Danas, K., & Hong, F. (2018). *Influence of the internal geometry on the elastic properties of materials using 3D-printing of computer-generated random microstructures*. SEG International Exposition and Annual Meeting, SEG 2018. Retrieved from https://doi.org/10.1190/segam2018-2998182.1

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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