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Isothermal solid-phase recombinase polymerase amplification on microfluidic digital versatile discs (DVDs)

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ABSTRACT

A new advance for massive DNA-based screening in limited-resource settings is demonstrated through the incorporation of easy-to-fabricate microfluidic chambers on digital versatile discs (DVDs) to perform isothermal recombinase polymerase amplification (RPA) in microarray format. Standard un-modified DVD discs and commercial drives are used for the low-cost detection method. DNA primers were printed in a microarray format on the polycarbonate surface of DVDs, with integrated control spots to guarantee the absence of false-negatives and false-positives. The solid-phase amplification assay, including the washing protocols and development reaction, was performed by dispensation of solutions through the inlet and the flow-movement controlled by DVD drive centrifugation. The final disc with reaction products was inserted into the DVD player and microarray images were captured and automatically processed. This simple approach was applied for the screening of genetically modified organisms (GMOs) in food samples. The limit of detection was 7 μg/g, well below the EU regulation limits for GMOs in food products. Hence, the only required materials for food safety monitoring were standard store-bought DVDs, plastic chambers, tips, pipettes, oven, and a standard DVD drive. The proposed strategy allows for an integrated microarray system with low-manipulation, reduced sample volume, and portable device applicable to low resource settings.

INTRODUCTION

The development of DNA biosensors is related to the adequate selection and integration of support, probes, assay format, and transduction phenomena to perform and detect the biorecognition. Unlike silicon chips, plastic polymers as analytical platforms offer the advantage of being transformed easy and cheaply into devices that join operations of sample treatment, fluid management, and detection.[1] But, in some cases, the proposed platforms are not useful for real application because the systems for fluid management and signal detection are not easily adaptable to wide-range of scenarios.

Several research groups and companies have been working in the development of biosensors based on the use of compact discs or 'lab-on-a-CD' systems. There are two main approaches depending on the nature of the disc used.[2] The first one includes plastic substrates with circular shape and several mm thick, which integrates a microfluidic system (microchannels, valves, chambers, etc.).[3,4] Some of them, known micro-total analysis systems (μTAS), integrate all analytical steps required for genomic assays.[5] Other lab-on-a-CD devices present a lower integration level and the procedure involves some handling steps.[6] In both cases, the measurement is generally performed with equipments, such as colorimeters, or expensive static detectors, such as fluorescence microscopes or other complex non-integrated systems.

The second approach uses directly audio-video compact discs as support for carrying out bioassays and the detection is based on the scanning of the focused laser present in conventional disc drives.[7] The main advantage of these technologies (CD, DVD, Blu-Ray and other) is mass-produced for the consumer electronic market with high-quality standards and cost-effective price. Our group has demonstrated that it is possible to use the CD player/writer as detector, using low-reflective discs (transmission/reflection mode) or conventional discs (reflection mode), also incorporating chemical modification of surfaces. These systems show higher sensitivity and working capacity (e.g. multiplexing), allowing the implementation of extremely inexpensive optical devices for biological applications.[8-10]

The development of DNA hybridization assays has been addressed using centrifugal disc platforms. The procedures include flow hybridization in different reservoirs, such as double-spiral,[11] channel,[12,13] or chamber,[14] and all of them combined with fluoresce detection. An interesting approach is the integration of isothermal amplification and fluorescent real-time detection with a commercially available centrifugal disc and analyzer.[6] Also, hybridization assays on microarray format have been performed based

on DVD,[9] and BD[10] technology (disc and detector). The experimental steps are similar to those when using glass or other solid supports. A distinguished advantage is that the use of expensive and bulky scanners typically imaging the spots is avoided, showing the way to the widespread of the microarray technology. Nevertheless, integrated approaches are required to reduce the number of steps and the manipulation of samples.

In a recent study, Santiago-Felipe *et al.*[15] has demonstrated the advantages of isothermal recombinase polymerase amplification (RPA) combined to DVD hybridization and detection by means of a technique called solid-phase amplification. In this approach, one primer is attached onto the polycarbonate surface of a DVD (bottom layer), while the other amplification components remained in the liquid phase. The polymerase extension of the primer produced a tethered and detectable amplification product. These results have opened a new strategy to integrate the amplification and the hybridization in the same platform at constant low temperature, avoiding devices with technologically complex heating/cooling systems. On the other hand, Russom *et al.*[16] have shown how merging optical discs and microfluidics holds a new step towards low-cost point of care applications. Low reflectivity DVDs were fabricated from 0.6 mm DVD substrates, including a spiral groove of 0.74 mm track pitch and coating with a 10 nm thick layer of SiO_2. The microfludic layers, containing microchannels and other fluidic reservoirs, were incorporated over metallic layer of discs (top layer). Then, the integration of the assay development, controlled by spinning rate, and reading (transmission mode) in only one platform was achieved. The system was applied to low-cost HIV diagnostics by counting CD4+ cells isolated from whole blood. However, this approach requires some disc modifications and the incorporation of a planar photodiode into the DVD drive to detect transmitted light.

In this study, a semi-automated DNA assay in microarray format is proposed based on the integration of a simple adhesive microfluidic layer on the polycarbonate surface of conventional DVDs (bottom layer). Primers are pre-printed onto the DVD surface, and lyophilized RPA reagents are stored within the spinning platform. The rest of the reagents are hand dispensed and the solid-phase amplification is performed, bringing the amplified products attached to the DVD surface. After the development of the amplification products, the microfluidic layer is removed and the disc is read by reflection mode (conventional DVD drive). The presence of the amplification product modifies the light intensity of scanning laser of DVD-drive (reflection mode), and using data acquisition software, a microarray image is generated. As proof of concept, the

method has been applied for the low-cost, reliable, rapid screening of genetically modified organisms (GMOs).

METHODS

Target genes. GMO testing was based on the determination of several genetic elements. Screening elements are the two most common transgenic genes (35S-promoter from cauliflower mosaic virus or p35S and nopaline synthase terminator or tNOS), allowing the detection of most of authorized or unauthorized lines.[17,18] Taxon-specific elements detect genes specific from plants, such as lectin (*Le1*) for *Glycine max* (soybean), alcohol dehydrogenase 1 (*adh1*) for *Zea mays* (maize), and LAT52 protein (*LAT52*) for *Solanum lycopersicum* (tomato). These elements increase the characterization of involved transgenic ingredients, allowing increase selectivity for certain GMOs.[19] Construction-specific elements are included for a complete identification of GMO events, such as Bt-11 construction, which involves a junction region between the intron 6 (IVS6) from maize alcohol dehydrogenase 1 gene (*adh1-1S*) and a synthetic *cryIA(b)* gene.[20]

Integrated DVD System Design. The DVD-based bioanalytical platform consists on two disc substrates (optical layer and microfluidic layer) bonded together (see supplementary information). The optical layer substrate used is a standard store-brought DVD-ROM disc purchased from MPO Iberica (Spain). According to the DVD specifications, a standard blank disc is composed of two 0.6 mm thick-polycarbonate substrates, with a middle layer of highly reflective metallic material (thickness 1.000 - 1.500 Å). The bottom polycarbonate layer has an injection molded spiral microguide (0.74 mm track pitch) in order to guide subsystems of the detector laser (λ = 650 nm) to be kept on the data track.

The microfluidic substrate was fabricated using 0.2 mm-pressure sensitive adhesive (PSA) (adhesive transfer tape 91022, 3M, USA) bonded to a disc-shape polycarbonate plastic (thickness 0.6 mm) with drilled access through-holes (diameter 1 mm). Ten identical fluidic structures, contained the microfluidic components, including channels, and reservoirs, were radially arrayed to enable multiplexed assays on a single disc using a CO_2 laser cutter (Hylax Hypertronics). A scheme of this device is shown in Figure 1, with details of the two chambers and channel locations. Each structure has a chamber

for the pre-amplification mixing (Fig.1A, Chamber 1) and a chamber for the solid-phase amplification and detection (Fig.1A, Chamber 2). The dimensions of the chambers are 5.5 mm in length, 5.5 mm in width and 0.2 mm in height, and so can contain a sample volume of 6 μL. The two chambers are connected by a 0.6 mm wide mixing channel as hydrophobic valve. The disc was designed to enable these simple fluidic steps at the low spinning rates (< 1500 rpm) achievable in commercial DVD drives.

Standard commercial DVD-ROMs were firstly conditioned by gentle ethanol washing, water rinsing, and dried by centrifugation. Biotinylated primers (Table SI.1) were immobilized on passively adsorbed streptavidin. For that, each mixture of streptavidin (5 mg/L) and biotinylated-labelled primer (100 nM) in 50 mM carbonate buffer, pH 9.6 and 1% glycerol (v/v), was printed on the polycarbonate disc surface (50 nL) with a non-contact AD 1500 BioDot Inc., CA printer. Working temperature and relative humidity were adjusted at 25°C and 90%, respectively. As this arrayer is traditionally used for printing on standard glass slides, a custom printing-layout was developed to print multiple arrays. The printing area for each chamber was 4 mm × 4 mm with allowance for minor misalignment with the printer. In a single run, 10 arrays of 9 spots (3×3) for primers, negative controls, and positive controls were spotted in the Chamber 2 region with a 1-mm track pitch. Pre-stored lyophilized reagents (0.8 mg) for amplification were dispensed into Chamber 1 with a spatula. The reagents mixture was composed by 2 mM DTT, 5% Carbowax 20M, 200 μM dNTPs, 3 mM ATP, 50 mM phosphocreatine, 100 ng/μl creatine kinase, 30 ng/ μl Bsu, and recombinase proteins (900 ng/μl gp32, 120 ng/μl uxsX, and 30 ng/μl uvsY) supplied by TwistDx (UK). Finally, the microfluidic layer was aligned to the DVD-ROM surface and affixed to enable a bubble-free flat disc. The outer holes were sealed with PCR sealer tape (Corning, USA) and stored at -20 ºC until use.

DVD drive. The assay performed on disc was controlled and measured by an adapted DVD drive from LG Electronics Inc. (Englewood Cliffs, USA). The device incorporated a data acquisition board (model DT9832A-02-OEM, Data Translation, Germany) with a sample rate up to 2 megasamples per second. A standard DVD drive has a motor to rotate the disc, an optical system with a laser (λ = 650 nm), and a servo focus/tracking system to centre and focus the beam on the spiral track. For the acquisition of data stored on the track, the laser scans the whole disc surface and reads the reflected intensity. Our device takes advantage of these components for two

objectives. First, the DVD drive is used as a centrifuge controlling the spinning rate of disc. Second, the drive is used as detector capturing the signal variation during surface scanning due to the presence of biochemical interaction (solid products).

The performances of the optical disc drive were controlled by custom software, written in Visual C++, running on a laptop connected to it through a USB2.0 universal serial bus interface. During the disk scanning, only signals coming from selected areas are processed for digitization, stored in the computer (5 MB size file). The signals for each track and microarray are deconvoluted into an image. The image analysis of microarray was also performed by the software.

Samples and extraction of genomic DNA. The certified reference materials (CRMs) were purchased from the Institute for Reference Material and Measurements (Belgium). Food products were bought in local stores. For genomic DNA extraction, aliquots of 5-20 g of homogenized sample were extracted using a GMO-extraction kit based on column purification, according to the manufacturers' instructions (Applied Biosystems, Spain). The extracted DNA was quantified by spectrophotometry (NanoDrop 2000/2000c, Thermo scientific Inc., USA) and stored at -20 ºC until analysis.

Amplification and development. Each sample was analyzed per quintuplicate, including positive and negative amplification controls. Solid phase RPA was performed on disc (10 reactions/disc, see supplementary information). Reaction mixtures (6 µL) contained 480 nM of each 5'-digoxigenin labelled primer (Table SI.1), 5 ng of genomic DNA, 14 mM of Mg acetate, and 1× rehydration buffer. Denhardt's reagent (2.5×, Life Technologies, Spain) was also added to reduce the non-specific background. Later, mixtures were dispensed with a micropipette on the corresponding microfluidic structure through the inlet holes. The outer hole was sealed with PCR sealer tape (Corning, USA). Then, the disc was inserted into the DVD player and a slow spin was done (<600 rpm for 10 s) to lead the reaction mixture to Chamber 1. After a proper reconstitution and mixing of the reagents, a spin was increased to 1000 rpm to fully move sample into Chamber 2 covering the pre-printed array. Next, the disc was introduced into a container (standard DVD plastic box) in a water-saturated atmosphere, and the solid-phase amplification reactions were carried out at 37 ºC for 40 min in an oven (model UF30 Memmert, Germany). After removing the sealer tape, the emptying of the chamber was done by spinning at 1000 rpm during 10 s. The dispensation of

solutions and reagents were done through the inlet, with the movement of solutions controlled by centrifugation as described above. The array was washed by dispensation of 0.1×washing solution (SSC, 1× saline sodium citrate: NaCl 150 mM, sodium citrate 15 mM, pH 7) and water through the inlet holes (6 µL). The chamber was emptied by spinning at 1000 rpm during 10 s. The detection was done using a mixture of anti-digoxigenin antibody produced in sheep (1/4000) and anti-sheep conjugated with horseradish peroxidase (1/500) in PBS-T (phosphate buffered saline and 0.05% (v/v) tween 20, pH 7.4) (6 µL). The developer reagent was 3,3',5,5'-tetramethylbenzidine (TMB) (6 µL). The array was washed with PBS-T plus deionised water as described above. Finally, the fluidic layer was removed and the disc was inserted into the DVD player.

DVD surface scanning. As the inner structure of the optical disc remained unaltered, the microarrays on surface were correctly read out. The disc was scanned at a rotation speed of 4× ≡ 13.46 m/s and the signal was acquired at 26 dB gain and 1700 Msamples/s, with a reading time lower than 10 min. Then, 10 microarray images were created (tagged image file format, grey-scale with 16 bit-colour depth, scale 0-65535). Optical intensity signals of each spot related with the amount of reaction product were quantified. In absence of a solid biorecognition product, the reflection properties of the disc surface were unchanged and the beam intensity collected by DVD drive was maximum, corresponding to the background signal of image. But, when the laser hit the deposit of TMB product, the intensity of laser beam that reached the photodiode decreased, corresponding to the signal of microarray spot. In this configuration, the mean intensity of each spot was from 1963 pixels (spot diameter 500 µm). After subtracting the local background, any spot displaying signal-to-noise ratio higher than three was considered as positive. The reading and image processing (feature gridding, addressing, segmentation, quality assurance) was automatically performed in less than 15 min by disc. Used discs were discarded following the same laboratory safety guidelines than ELISA plates.

Complementary measurements. Photos of the fluidic process, shown in Fig.1 (B-E) were taken using a custom visualization stand composed by a motor (EC-Max 40 Series DC) and controller (ESCON 5/50) supplied by Maxon (USA) and CMOS camera (uEYE camera model UI-3360CP) supplied by IDS (Germany).

Amplification products recovered from the reaction chamber were checked by electrophoresis on a 3% (w/v) agarose gel at 110 V and room temperature. Gels were stained for 30 min with 0.5× TBE buffer (Tris/Borate/EDTA) containing fluorophore RealSafe (Real Laboratories, Spain) at 0.01% (v/v), and bands were visualized with a UV transilluminator. Product size was determined by comparison with a 50 bp ladder (Fermentas, Lithuania).

RESULTS AND DISCUSSION

Microfluidic DVD characterization. A simple system was designed composed by a reaction/detection chamber (microarray) connected by a channel to a loading chamber for the dispensation of reagents (Figure 1). Fluid propulsion on the DVD disc platform was achieved through centrifugally-induced pressure on the fluid as the disc spins.[21] The flow rate was dependent mainly on the rotational speed of the disc, the location of the fluidic, the geometry of the fluidic channels, and the specific fluidic properties (density, viscosity, and surface energy). Through the utilization of combinations of different channel geometries and spin speeds, precise flow rates ranging from nanoliters to millilitres per second were modulated. The fluidic valves were designed for use in the 0-1500 rpm range to allow for easy automation within a standard DVD drive.

Process monitoring was performed with a custom visualization stand for the optimization of the process. Regard to the reagent loading, the images showed that the fluidic samples were perfectly introduced into loading chamber (Chamber 1 of Fig. 1C) and held in place via a capillary valve. A critical challenge studied in the development of an integrated system was the storage of amplification reagents and their release. The microfluidic structure included a hydrophobic valve to allow for the rehydration of reagents after sample loading, and the active life of the in-disc reagents was verified for at least two weeks.

After the initial set of tests, a serpentine mixing channel was designed to ensure complete mixing of the lyophilized reagents with the inputted sample before reaching reaction chamber. The selected dimensions were a width of 600-μm and a total linear length of 38 mm, containing six elbows. With this configuration, the capillary valve was open when the disc was spun at 2000 rpm for 20 seconds, and the sample was fully transferred into reaction chamber (Chamber 2 of Fig. 1D and 1E). The entire reaction

chamber was fully covered by solutions. No formation of bubbles or other artefacts was observed.

In addition, the microfluidic structure was designed for washing the reaction chamber controlled by disc rotation. To characterize the optimum washing speeds, the flow rate through the structures was modelled using the governing equation [Kellogg]:

$$Q = A \cdot \frac{D_h^2 \rho \omega^2 \bar{r} \Delta r}{32 \mu L}$$

where Dh is the hydraulic diameter of the channel, ρ is the liquid density, ω is the angular velocity of the spinning CD, is the average distance of the liquid element from the CD centre, $\bar{r}$ is the radial extent of the fluid, Δr is the fluid viscosity, and L is the length of the liquid in the channel. The hydraulic diameter of the channel, D_h, is defined as 4A/P, where A is the cross-sectional area of a rectangular channel and P is the wetted perimeter of the channel. In repeated trials using the 600-μm serpentine channel, the washing liquid was fully transferred in 6 seconds at 500 rpm, corresponding to a flow rate of 1 μL per second. This result closely matches the calculated theoretical flow rates given the channel design specifications.

Optimization of the solid-phase RPA format. The isothermal solid-phase amplification was addressed according to the assay recently published based on a heminested mechanism.[15] It consisted on immobilising primers, following a microarray layout, on the support of reaction chamber. Other amplification components in liquid phase were dispensed from the loading chamber. After the reaction chamber was filled, the amplification was produced in static mode, i.e. no rotation during reaction. As unbound primers were added to the reaction mixture, the amplification was produced in both phases (in liquid and on the surface). Then, centrifugal microfluidics was just used for the semi-automated reconstitution of lyophilized reagents, mixing with samples and dispensation minimizing the contamination and increasing reproducibility of assays. Nevertheless, merging DNA assays on microfluidic discs required the selection of a robust chemistry able to resist the solution flow. Three processes – sensitive to flow action – were studied: (i) immobilization of primers, (ii) washing protocols, and (iii) developing reaction.

In regards to primer anchoring, an indirect adsorption mode was chosen based on the streptavidin/biotin recognition. The main advantage was the simplicity of process

because microarraying of biotinylated primers and streptavidin was directly performed on bulk discs without previous surface treatment of DVD or blocking steps. Under these working conditions, immobilized primers were resistant to a flow up to 13200 nL/s (at 2000 rpm). On the other hand, the amplification process on solid supports depended on the immobilization density of the primer. To that end, coating conditions were optimized by varying the streptavidin concentration from 5 to 20 ppm, and the primer concentration from 50 to 200 nM. The highest signal was obtained for a primer concentration of 100 nM (0.06 fmol/mm^2) (Figure 2A).

The set-up of post-amplification steps was important in the integration of the assay in the microfluidic device. Comparing to PCR, RPA mixture is a high-viscosity solution with a higher number of components, making the washing protocol more crucial. After the solid-phase amplification, the rest of reaction components must be effectively removed without the release of the products immobilized on disc surface of reaction chamber. The composition of washing solutions was optimized in conventional DVDs – without microfluidic structures – controlling parameters such as pH, ionic strength and astringency. SSC buffer and PBS-T buffer were selected for washing after the amplification process and after the antibodies incubation, respectively. In microfluidic discs, two protocols were assayed for both the post-amplification and the post-antibody incubation steps. In the static protocol, referred as 0 rpm, the washing method was based on completely filling the array chamber, incubation, and a fast removal of the liquid. In the in-flow washing method, the washing buffers passed continuously through the array (disc spin 0-1500 RPM). Since the in-flow washing protocol decreased significantly the signal, the static protocol was chosen (Figure 2B). Then, the washing cycles (1-5) were studied for both washings (post-amplification and post-antibody incubation). Figure 2C shows that a higher number of washing cycles produced a significant decrease in the signal due to the product releasing.

Two signal enhancement reactions were compared for developing the digoxigenin-labelled products immobilized in the reaction chamber. First, metallographic reaction was based on the dispensation of anti-digoxigenin antibody produced in rabbit (1/7500 dilution), and anti-rabbit antibody conjugated with gold (1/100 dilution), and using silver as developer reagent. Second approach consisted on enzymatic reaction dispensing anti-digoxigenin antibody produced in sheep (1/4000 dilution), and anti-sheep conjugated to horseradish peroxidase (1/500 dilution), and followed by the addition of TMB as developer reagent.

Both reaction sequences (metallographic and enzymatic approaches) produced a detectable precipitate that modified the laser intensity of DVD drive (λ= 650 nm) during the disc scanning (Figure 2D).

However, metallographic reaction provided high and variable background signals, which results in low and irreproducible signal/noise ratios and false-positives, even increasing the number of washing cycles. The unusual high background signal of metallographic developing may be due to an incomplete washing of components present in the RPA mix (Carbowax, proteins, etc.) or developing reagents, i.e. antibodies, could enhance the reduction of silver, leading to nonspecific depositions. In the enzymatic reaction, any nonspecific signal was observed after a single washing cycle. Since the enhancement reaction using TMB was less sensitive to the rest of RPA components, the enzymatic reaction was selected.

Analytical performances for GMO detection. The method was applied for the screening of GMOs including screening, plant-specific, and construction-specific elements. The increasing production of transgenic crops and the concern regarding the safety of derived foods has led to the extensive monitoring of foodstuff that could contain GMOs. Hence, the development of low-cost, reliable, rapid analytical methods for their detection and quantification through the entire production chain is of great importance.

The sensitivity of the assays was determined in two ways, by analyzing serially diluted genomic DNA (10-folds dilution) and by analyzing samples with different concentrations of transgenic ingredient. For the first approach, concentrations of DNA dispensed to the inlet chamber varied from 100,000 µg/g (10 %) to 0 µg/g (0 %). An excellent correlation between the concentration of transgenic template and the optical intensity measured by DVD detector was found ($R^2 = 0.976$) (Figure 3).

For the second approach, sensitivity of the method was assessed by simultaneously determining samples with decreasing concentrations of transgenic foods in relation to non-GMOs foods, from 0 % (w/w) to 10 % (w/w). A t-test revealed that there was no significant difference between the slopes of the calibration curves (p-value=0.806>0.05).

The detection limits (LODs) were calculated as the lowest amount of DNA able to produce a signal that can be distinguished from the blanks (NTC: control solution

without template or food without transgenic ingredient). All target genetic elements were detected at a concentration of 110-460 μg/g (0.011-0.046 %).

The estimation of the sensitivity of the method in term of copy numbers can be made theoretically as described in reference 20. Taking into account the genome size of the plants and considering them in their haploid form, 100 ng DNA would contain 36630 copies of genome for the maize and 88496 copies for the soybean. Then the limit of detection of 0.01% GMO in that amount of genomic DNA (100 ng) would then correspond to about 3.7–8.8 haploid copies of target sequences for the different plant species. These results are similar or better than others obtained by RT-PCR (1-16 copies)[22] or by PCR-microarray (37-88 copies).[20] Thus, the system can reliably comply with the legal requirements of the 0.9 % limit of detection of the EU.

Assay reproducibility, expressed as relative standard deviation (RSD), was determined from the optical density of spots from samples analyzed in triplicate each one in three DVDs. The intra-day RSD varied from 3.0 to 6.7 %, and the inter-day RSD from 5.4 to 11.4 %.

Detection of GMOs in certified reference material and food samples. The capabilities of method were evaluated by detecting GMOs in 7 certified reference materials: maize Bt176 (ERM-BF411), maize Bt11 (ERM-BF412b and ERM-BF412f), maize GA21 (ERM-BF414b), maize MON810 (ERM-BF413ck), maize Bt176 (ERM-BF411) and Roundup ReadyTM Soya (ERM-BF410dk). All the elements of the different GMO were detected and they corresponded to the expected pattern (Table 1). The four lines of transgenic maize provided positive responses for maize-specific gene (*adh1 gene*) and negative for the rest of taxon probes. The transgenic line of soybean (RRS) provided positive responses for soybean-specific gene (*Le1 gene*) and negative for the rest of taxon probes. Screening elements and construct-specific elements were correctly detected in all cases and no cross-contamination between adjacent chambers was observed (n = 50). A sample was considered positive when the optical response was higher than the cut-off value (optical density of 2550).

The method was also applied for the detection of food samples. Figure 4 shows an example of the optical signals registered by DVD drive. As it can be seen, samples containing GMO ingredients were detected because positive responses were observed in the corresponding spots. Table 1 proves that positive results were observed in all cases for the analytes declared, even at trace levels, or in spiked samples. Negative results

were found in most of the samples declared to be analyte free. The only exception was cookies in which, despite not having declared any GMO, positive results for the p35S analysis were obtained. It can be explained because its concentration should be lower than 0.9% (EU-regulation).

The reliable and sensitive results achieved indicate that the proposed method is useful for GMO detection in routine food-safety monitoring.

CONCLUSIONS

Screening protocols requires analytical platforms with properties such as high working capacity, sensitivity, and reliability. This study is aligned to recent researches focused on merging optical discs and centrifugal microfluidics to address a new step towards an increased automatization, reduced sample consumption and low cost diagnostics. The proposed system integrates microfluidic chambers on digital versatile discs (DVDs) to perform an isothermal DNA solid-phase amplification in microarray format. The novelty is that microfluidics is in the bottom layer of the DVD and the measurement is performed by reflection using a standard DVD player (small dimensions, lightweight, and connectable to internet or telephone network device). Developed discs, due to their properties such as high mechanical resistance, good thermal stability and hydrophobicity, have demonstrated to be an excellent option as bioanalytical platform. The prospective costs of the system (<2 € disc and <500 € reader) are below the state-of-art, i.e. qPCr plates and fluorescence-thermocyclers. In addition, the method is easy to operate by locally trained staff, and requires inexpensive and unspecific equipment (extraction columns, pipettes, oven, DVD drive, and laptop). The properties of the proposed system make it suitable to be applied in a wide-rage of ambits such as low resources settings, satellite/decentralized laboratories and production plants.

As proof-of-concept, the device was applied to the detection of GMOs being their reliable identification an important issue because the labelling is legally regulated. Screening methods are especially required due to the high number of samples and genes to be controlled. This low-cost technology for semi-quantitative analyses has shown excellent analytical performances (selectivity, sensitivity, reproducibility, and high throughput). The integration of the amplification and hybridisation steps in a one-pot reaction allows the processing of the samples in less than 90 min, reducing the manipulation, the reagent consumption, and the risks of cross-contamination. Despite

the simplicity of the approach, the results demonstrate that this screening assay can be applied without compromising analytical performance and that it well suits routine genomic analysis.

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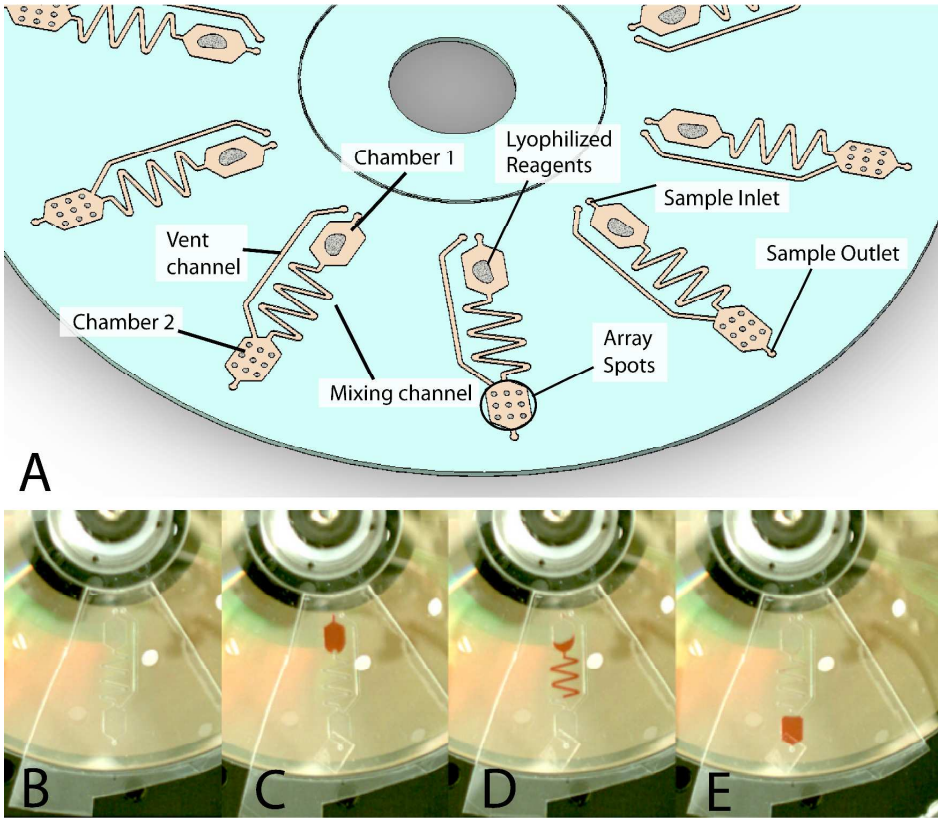
522 Figure 1.



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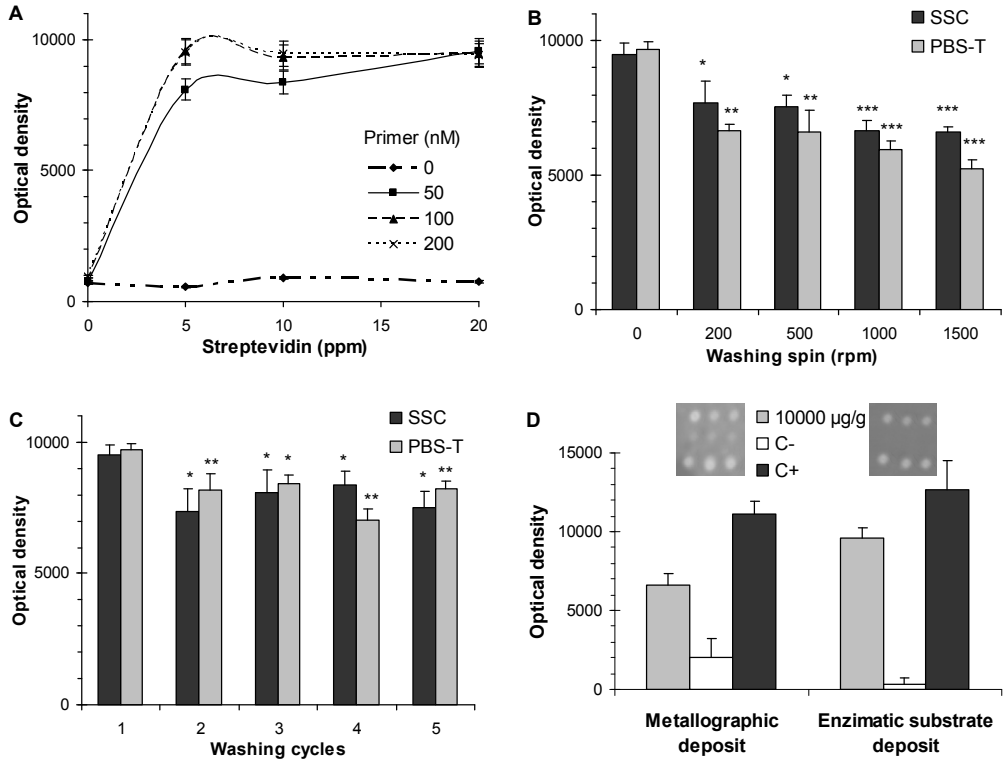
528 Figure 2.



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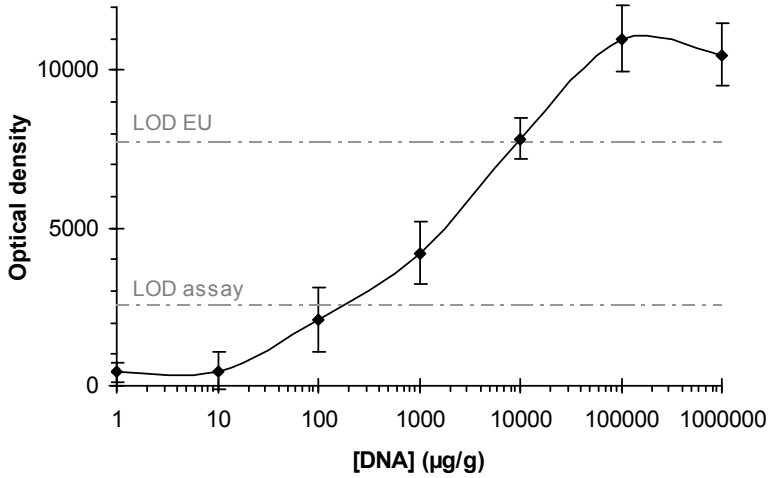
533 Figure 3.



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537 Figure 4.

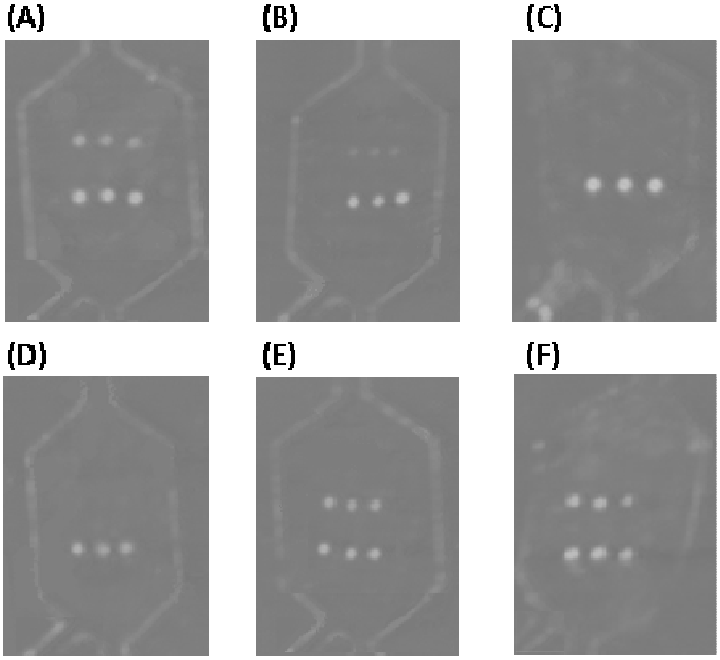

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Table 1.

Sample	Certified/Declared GMO content	DVD Detection					
		Taxon			Screening		Construction
		Le1	*adh1*	*LAT52*	p35S	tNOS	Bt-11
CRM Bt-176 maize	2.00 ± 0.11 %	-	+	-	+	-	-
CRM Bt-11 maize	0.98 ± 0.29 %	-	+	-	+	+	+
CRM Bt-11 maize	4.89 ± 0.21 %	-	+	-	+	+	+
CRM GA21 maize	0.10 ± 0.08 %	-	+	-	-	+	-
CRM MON810 maize	5.00 ± 0.11 %	-	+	-	+	-	-
CRM RRS soya	10.00 ± 0.10 %	+	-	-	+	+	-
Tomato NahG	Presence	-	-	+	++	++	-
Feed	Presence	-	+	-	++	++	++
Cookies	No declared	+	+	-	+	-	-
Ketchup	No declared	-	+	+	-	-	-
Soy sauce	No declared	+	-	-	-	-	-
Soy sauce+RRS	0.5%	+	-	-	+	+	-
Sweet corn	No declared	-	+	-	-	-	-
Sweet corn+MON810	0.1%	-	+	-	+	-	-
Cereals baby food	No declared	-	+	-	-	-	-
Noodles	No declared	-	-	-	-	-	-


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