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One-pot isothermal DNA amplification-hybridisation and detection by a disc-based method

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ABSTRACT

An integrated sensor comprising isothermal DNA amplification and *in situ* detection is presented. The method principle is based on recombinase polymerase amplification (RPA) and detection in the microarray format by compact disc technology as a high-throughput sensing platform. Primers were immobilised on the polycarbonate surface of digital versatile discs (DVD) and, after hemi-nested amplification, multiplexing identification of each tethered product was achieved by optical scanning with a 650 nm-laser of the DVD drive. The efficiency of one-pot hybridisation/elongation/detection depended strongly on probe density and other factors such as the concentration of the unbound primers present in solution. The optimised conditions provided equivalent amplification factors (7.3×10^8 – 8.9×10^8 fold) to those obtained by conventional reactions performed in vials. The proposed method was applied to *Salmonella* detection (generic by *hns* and *oriC* genes, and specific for subspecies I by *STM4507* gene). A triplex assay was satisfactorily compared to the non-integrated protocols. Food and vaccine samples were analysed in a shorter time with less handling. The results indicate that the multiplex DVD assay is a simple, competitive, isothermal, portable system that is particularly useful for microbiological routine analysis.

Keywords: isothermal solid-phase amplification, compact disc, pathogen, microarray

INTRODUCTION

The detection of specific DNA targets is interesting in genetic or infectious diseases diagnosis, bioterrorism, food safety and forensic areas. Traditionally, polymerase chain reactions (PCR) combined with techniques such as gel electrophoresis or solid hybridisations, have been used to reveal the presence of single-copy genes. However, these two-step protocols can also lead to sample contamination [1].

Different methods have been proposed for the integration of amplification and hybridisation. The most relevant strategy is the use of real-time PCR, which allows the rapid, quantitative identification of DNA targets through the use of specific probes. Yet expensive non-portable instrument is required for sensitive fluorescent measurements, which restricts this technique for point-of-need applications. This limitation has been overcome with the application of microfabrication technologies integrating PCR reactors in a microfluidic device [2-3]. However, these solutions are useful for the simultaneous determination of a small number of genes.

Sensors based on integrated microarrays seem to be an ideal approach since their miniaturised size allows the arrangement of numerous probes in a relatively small space and reaction volume. An interesting category is lab-on-a-chip systems that incorporate different connected chambers for each analytical step, including one for hybridisation in a microarray format [4-5]. In these systems, the control of changes in evaporation or temperature is crucial because performance might be affected [6].

A technique called solid-phase PCR was developed for the integration of both amplification and hybridisation processes in a single step [7-10]. To this end, one or both primers is/are attached on a solid support (beads, microplate, or a microarray format on a flat surface), while the other PCR components remain in the liquid phase. Enzymatic extension of the primer directly produces a tethered amplification product. The advantages are high throughput, ease of operation, and specific fast detection. However, amplification yields are lower than those of conventional solution-phase reactions [8]. It has been demonstrated that the addition of forward and/or reverse primers to the reaction mixture can enhance amplification efficiency because the reaction proceeds in both the liquid and solid phases [9]. When the attached primers differ from those in solution, the mechanism is called heminested solid-phase amplification. Here the amplification of an extended nucleotide sequence is followed by the amplification of a region located within the first amplification product, which provides improved selectivity and sensitivity [11].

The throughput of the above-discussed PCR-based methods is limited by thermal constraints because an imprecise temperature leads to lower amplification efficiency. In the last few years, two approaches have been considered to overcome this drawback. Firstly, the development of devices with accurate temperature control and fast transitions between stages, using low thermal conductivity materials [5,12]. Secondly, the use of other polymerases (or combination of enzymes) allows for isothermal amplification, thus simplifying the heating device [13]. In the latter option, there are some relevant examples based on strand displacement amplification (SDA), helicase-dependent amplification (HDA) and recombinase polymerase amplification (RPA), which have been combined with detection by silicon microring resonators, on-chip fluorescence, electrochemical devices or lateral flow strips [8, 14, 15, 16, 17]. However, these methods are performed far from room temperature (60-65ºC), require a time-consuming step for surface treatment and functionalisation, or need a complex fabrication process of devices and expensive detectors.

The aim of this study was to develop an integrated system that provides both the flexibility of isothermal amplification and the multiplexed capacity of microarrays in a homogenous assay. According to our experience, in-tube RPA is readily compatible with subsequent solid-hybridisation. The studied one-pot approach involves the development of the solid-phase RPA reaction in a microarray format onto the sensing surface. As proof-of-concept, the assay is done on an optical low-cost portable analytical device and does not use microfluidics. Hybridisation/elongation is performed on the surface of a digital versatile disc (DVD) and the products are detected with a standard DVD drive [18-19]. The principles and benefits of this innovative simple sensor are showcase through detecting *Salmonella* bacteria in different clinical and food products. Nevertheless, this strategy can be easily extended to other targets, layouts and detection systems.

METHODS

DNA targets. The assay was developed for the simultaneous detection of three genes: *hns* and *oriC* for the generic identification of *Salmonella* spp., and *STM4057* for the specific detection of *Salmonella* subspecies I. The primers used in *hns* gene detection were 5′-digoxigenin- TACCAAAGCTAAACGCGCAGCT-3′ (forward), 5′-TGATCAGGA-AATCTTCCAGTTGC-3′ (solution reverse), and 5′-biotin-TEG-T_{10}-TTTGATTA-CAGCCGGTGTACGACCCT-3′ (surface reverse). The primers used in *oriC* gene detection were 5′-digoxigenin-TTATTAGGATCGCGCCAGGC-3′ (forward), 5′-AAA-GAATAACCGTTGTTCAC-3′ (solution reverse), and 5′-biotin-TEG-T_{10}-GCTAGTG-ATCCTTTCCAACGCATTG, (surface reverse). The primers used in *oriC* gene detection were 5′-digoxigenin-GGTGGCCTCGATGATTCCCG-3′ (forward), 5′-CCCACTTG-TAGCGAGCGCCG-3′ (solution reverse), and 5′-biotin-TEG-T_{10}-GCCCGGCCTCC-GGTGAAGGTAATTT-3′ (surface reverse). Food and clinical samples were analysed. Inoculation assays were also prepared by adding 10-fold serial dilutions of an 18-hour culture of each pathogen in sterile saline solution (0.8% NaCl) covering a range from 0 to $4 \cdot 10^4$ colony forming units per millilitre (CFU/mL). The protocols for DNA extraction, the description of the cells and the other oligonucleotides used in this study are available in the Supplementary Information.

Microarraying. Recordable DVDs were purchased from MPO Iberica (Spain). Discs were firstly conditioned by gentle ethanol washing, water rinsing, and dried by centrifugation. Primers were immobilized by passive adsorption by means of streptavidin-biotin interaction. For that, each mixture of streptavidin (10 mg/L) and biotinylated-surface primer (50 nM) in printing buffer (50 mM carbonate buffer, pH 9.6 and 1% glycerol (v/v)) was transferred to the polycarbonate disc surface (50 nL) with a non-contact printer (AD 1500 BioDot Inc., CA, USA), and the working temperature and relative humidity were controlled at 25°C and 90%, respectively. Different microarray layouts, or distributions of primers on the surface, were examined (Supplementary Information). The final layout consisted of 36 arrays of 25 dots each (5×5) with a 1-mm track pitch: four spots corresponding to *hns* gene, four dots corresponding to *oriC* gene, four dots corresponding to *STM4057* gene, four positive controls, and nine negative controls (immobilisation and hybridisation).

Immobilisation densities of primers were determined by using a dual labelled oligonucleotide (biotin at the 5'-end and Cy5 at the 3'-end), and fluorescence was measured by a homemade reader device. Excitation of the Cy5-marker was achieved by light emitting diodes (Toshiba TLOH157P) at an angle of 55º, illumination of 150 mW and wavelengths of 595-615 nm. Emission intensity was collected by a high sensitivity charge-coupled device (Qimaging Inc., Canada). Then, the amount immobilised on the disc was estimated from a calibration curve obtained from serial dilutions of the Cy5-labeled oligonucleotide (0.1–100 nM).

Integrated procedure. The amplification enzymes, nucleotides and buffer (TwistAmp Basic from TwistDx, UK) were mixed with 160 nM of both primer pairs (forward and reverse solution primers) for the three target genes and 10 ng of genomic DNA, extracted sample in triplicate. Denhardt's reagent (2.5×, Life Technologies) and inert oil (8%, v/v, Sigma) were also added to reduce the non-specific background and to generate an inert overlay for minimising the evaporation phenomenon, respectively. Later, mixtures (25 μL) were dispensed with a multi-channel micropipette to form a spherical sessile droplet on the corresponding microarray layouts. Positive and negative amplification controls were also included.

The droplet dimensions were measured using a Dino-Lite Digital Microscope (BigC.com, CA) with a resolution of 1.3 megapixel (1280×1024 pixels). Accordingly, the volume of droplets was established [eq. 1] by three parameters: contact radius (r), droplet height (h), and contact angle (θ).

$$V = \frac{\pi}{6} h\left(3r^2 + h^2\right) = \frac{\pi r^3}{3} \frac{f(\theta)}{\sin^3 \theta} \qquad \text{[eq. 1]}$$

where $f(\theta) = 2 - \cos\theta\,(3 - \cos^2\theta) = (2 + \cos\theta)(1 - \cos\theta)^2$.

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 37ºC for 40 min in a heating oven. The immobilised product was developed according to the protocol described in reference [18].

Sensing principle. The amount of pathogen was related to the optical density of the reaction product, which was read directly by an adapted DVD drive from LG Electronics Inc. (Englewood Cliffs, NJ, USA) and controlled by custom software, with

a reading time lower than 10 min [18]. The measurement principle is based on the optics of the DVD drive and the variation of the reflection properties of the DVD surface given the presence of the biorecognition product. Briefly during DVD scanning following the disc track (rotation speed 4× ≡ 13.46 m/s, 26 dB gain, 1,700 Mega-samples/s), the laser strikes the product which attenuates laser beam intensity that reaches the photodiode of the pickup. If there is no solid product, the reflection properties of the DVD surface remain unchanged and the maximum intensity of reflected beam is collected by the DVD drive (background signal). The analogue signals are acquired directly from the photodiode of the DVD drive. Then grey-scale images (the tagged image file format, colour depth 16 bit, scale 0-65535) were generated and the optical intensity signals of each spot were quantified using in-home software. Image processing (feature gridding, addressing, segmentation and quality assurance) was automatically performed in less than 5 min per disc.

Conventional procedure. In the first step, triplex RPA reactions were performed in vials (25 μL) by adding 160 nM of each primer and 10 ng of the genomic DNA from extracted sample to the reconstituted RPA solution. Furthermore, deoxynucleotides (200 μM) were added to obtain the correct amplification yield for the multiplex assay. Tubes were placed inside a heating oven (Memmert UF30, Germany) at 37ºC for 40 min. In the second step, the hybridisation assays on the disc were carried out with 10 μL of the amplified product mixed with 90 μL of 5× hybridisation buffer (1× saline sodium citrate, NaCl 150 mM, sodium citrate 15 mM, pH 7) containing 10 nM of the positive hybridisation control. Subsequently, the solution was denatured by heating at 95°C for 5 min and transferred to the microarray on the DVD polycarbonate surface. After 40 min of incubation at 37ºC, the array was washed with pure water. Products were developed and imaging was performed as described above.

Data processing. The amplification efficiency (*E*) in the PCR, or the increase of product per cycle, might be described by the equation $E = (N/N_0)^{1/n}$, where N_0 and N are the initial and final number of targeted molecules, and n is the total number of cycles. *E*-values vary between 1 and 2 per cycle. For the RPA (isothermal amplification reaction), efficiency was associated with the average number of primer extensions by *Bsu* DNA polymerase I. Therefore the *E*-value, or the average increase in product by the time unit,

was calculated from the global amplification yield described by the equation $E = (N/N_o)^{1/t}$, where t is the reaction time (in minutes).

The statistical package SPSS for Windows v. 16.0 was used for the data analysis of the optical signals.

RESULTS AND DISCUSSION

Droplet format. A first study to establish the behaviour of the reaction droplets on the sensing surface was developed by dispensing the RPA mixtures on DVDs. The high hydrophobic nature of polycarbonate generates spherical sessile droplets (Figure 1). The statistical analysis confirmed that the right and left contact angles were the same ($F=0.04<F_{1,6}=5.99$, p-value>0.05).

Several factors were studied to reduce droplets evaporation due to the gas/liquid equilibrium. The variation of the buffer composition ($MgCl_2$ range 5-20 mM, formamide range 0-25%, Tween range 0-0.5%) did not yield satisfactory results. However, the water saturated ambient (DVD box) and the presence of inert oil in the reaction mixture (8% v/v) forming an external lipid layer, reduced water evaporation. Regarding volume, small droplets increased the assays per disc and cut the analysis cost, but a poor response was produced because the amplification reaction was partially inhibited. Hence, a droplet volume of 25 μL was selected, with 5.16 ± 0.02 mm being the diameter on the polycarbonate surface. Under these conditions, the evaporation regime in accordance with temperature (25-55ºC) was established (Figure 1). The drop volume diminished linearly with time, just as Pittoni et al. [20] reported for the polycarbonate surface. The initial droplet had a contact angle of 59.5 ± 1.4º, a height of 1.72 ± 0.04 mm, and a contact radius of 2.87 ± 0.12 mm. After 40 min heating in an oven at 37ºC (the working temperature of the RPA), the contact angle and height were reduced to 43.1 ± 0.5º (-27%) and 1.14 ± 0.02 mm (-38%), respectively, but increased the contact radius to 2.95 ± 0.04 mm (+3%). These changes in the droplet dimensions (< 20% in volume) did not modify the polymerase activity.

An assay with 36 droplets was chosen (Figure 1). The positions of the arrays were chosen to directly dispense the reagents with a multi-channel micropipette (distance between flanking droplets: 1 cm). Moreover, the hydrophobic nature of the sensing surface (polycarbonate, contact angle of ~ 90°) allowed correct platform manipulation

without any cross-contamination effect between the adjacent droplets. The available area for each microarray was 20.9 ± 0.2 mm^2.

Optimisation of the solid-phase RPA format. Different solid-phase amplification approaches were studied to check the absence of false-positive or false-negative responses and to compare spot intensities.

The first approach was based on immobilising primers on the solid support and on dispensing other reaction components in the liquid phase onto the disc. The results obtained were very poor in terms of sensitivity (signal-to-noise lower than 5 for 2 ng DNA/mL). In a second approach, the addition of unbound primers to the reaction mixture led to amplification in both phases (in liquid and on the surface), and gave satisfactory results (signal-to-noise higher than 10 for 2 ng DNA/mL). The liquid fraction analysis confirmed the presence of the amplification product.

Spot intensities depended on the immobilisation density of the primer. To this end, different amounts of reverse primer were anchored on the surface. The effect of the attached primer concentration (immobilisation densities from 0 to 0.13 fmol/mm^2) to the optical signal is plotted in Figure 2A. The highest signal was obtained for 50 nM (0.03 fmol/mm^2). Higher concentrations led to a decreased signal, probably due to the steric effects; i.e., hindrance, repulsion, etc., between attached primers and/or amplified products, as demonstrated for solid-phase hybridisation on glass surfaces [10].

The effect of immobilising a single primer (asymmetric amplification) or both primers (bridge amplification) onto the solid surface was also assessed (Figure 2B). The analysis of variance (ANOVA) test showed that there was no significant difference attaching the forward primer, the reverse primer, or both ($F=2.1<F_{2,12}=3.89$, p-value>0.05). For further experiments, the reverse primer was immobilised. As a negative control, a primer with a dideoxy nucleotide at the 3′ end was used to avoid primer elongation. Since the signal was comparable to the background, non-specific hybridisation processes were done. Thus the optical signal recorded with conventional primers was completely due to the solid-phase amplification.

The concentration and nature of the unbound primers, that is, those added to the amplification mixture, had a strong effect on response (Figure 2C). Firstly, the addition of the reverse primer led to an incremented signal in accordance with concentration, with the highest value reached at the stoichiometric rate if compared to the forward primer. The interpretation result was based on asymmetric amplification efficiency, as has been described for in-solution PCR [21]. If a reverse primer was lacking in the

solution, the forward primer/reverse primer ratio was very high due to the immobilisation density of the attached reverse primer (0.03 fmol/mm^2). In this case, the estimated *E*-value was close to 1 (linear amplification) as only one strand, corresponding to the forward primer, was available to be used as a template. Yet for the quasi-stoichiometric proportion between primers in the solution, both strands were potentially available. Then the estimated *E*-value obtained higher values (exponential amplification) and the maximum response was achieved.

Figure 2C also shows the results for the two solid-phase approaches according to the unbound reverse primer sequence if compared to the attached one. Conventional amplification (sp-RPA) involved the formation of the same product in the liquid phase as in solid phase, because the same sequence is used for both phases. Meanwhile, the heminested approach (heminested sp-RPA) led to a shorter immobilised product than that formed in the liquid phase, because the attached primer was designed to be specific for a region located within the first product. Then the initial amplification (in solution), was followed by the solid-phase nested reaction. No signal difference was observed when the reverse unbound primer was equal to (sp-RPA) or differed from (heminested sp-RPA) the attached primer on the surface and, consequently, similar *E*-values were reached. The heminested approach was chosen because this format guarantees higher selectivity since three specific regions of the target gene are involved in the assay.

Our previous vial-RPA studies have demonstrated that as product length increases, the number of copies lowers and the reaction rate improves due to enzyme processivity; i.e., measurement of the global number of nucleotides added per time unit. In the heminested sp-RPA, a similar effect was expected because DNA polymerase elongated both primers (solution and surface). Then, the size of both reaction products was studied by changing the forward and/or the reverse primer used, which led to product lengths of between 87 bp and 152 bp for the solution-products, and of between 53 bp and 106 bp for the surface-products (Figure 2D). The ANOVA test showed that the signals for the one-pot format were comparable ($F=0.877<F_{3,\ 12}=3.49$, p-value>0.05). These results indicated that enzyme processivity affected the global amplification yield, but these variations were not observed on the surface reaction.

AFM imaging. The AFM imaging of DVD surface revealed changes in the topography of the surface, as consequence of the biomolecules immobilization (Figure SI.6). Raw polycarbonate exhibited a smooth and featureless surface, with a maximum peak depth at 1.34 nm. After primer immobilization, by means of streptavidin-biotin interaction, a

homogenous spread of globular particles appeared. The analysis of the dimensions of these particles reported a minimum diameter of around 11 nm, corresponding to the streptavidin as it has been previously described [22]. After the solid-phase amplification, an increase of the height was observed from 6.3 to 6.8 nm. This variation may be due to two effects related to the amplification reaction, an increase of the density of the attached molecules and the presence of double-strand DNA [23]. Previous studies have reported similar height increments for the hybridization of a 150 pb amplified product [22].

Effect of temperature and reaction time. Figure 3 shows the optical signal with the temperature and reaction time for the integrated reaction when compared to the optical signal recorded by the two-step protocols. The maximal response for the heminested sp-RPA format was accomplished in 40 min at 37ºC, but the signal was approximately 20% lower than that obtained when amplification and hybridisation took place separately in a vial and on a disc, respectively. This decrease is in agreement with those reported in previous studies based on solid-phase PCR, which shows that the amplification rate is lower than for the two-step protocols [11]. This fact can be explained if we consider that the high negative charge density of the immobilised primers on the solid support may disturb polymerase functioning on the surface, and/or may repel the target DNA in solution, to reduce hybridisation and extension efficiency. Additionally in the solid-phase formats, the reagents (enzyme, nucleotides, and especially the DNA template) have to be transported to the surface before elongation from the attached primer starts. Subsequently, a concentration gradient is formed between the bulk solution close to the surface, which diminishes amplification efficiency (limiting reactant).

The heminested sp-RPA format proved more sensitive to temperature than the amplification in the vial. This worse temperature tolerance (5-fold lower) might be due to the reagent concentration variations caused by the droplet evaporation process, rather than by modified enzymatic activity. Then different heating systems were examined to control the isothermal process. Assay performance was evaluated using several low-cost heating devices: oven, infrared lamp, hot plate, water bath. The obtained droplet volumes and optical signals are shown in Table 1. The ANOVA test was performed and showed two groups of devices if compared to signal intensity. Higher responses were achieved for the non-contact heating systems (oven and IR lamp), if compared to systems based on heating by contact ($F=17.58>F_{4,5}=5.19$, p-value<0.05). These

differences are explained by thermal conductivity (λ=0.241 $W \cdot m^{-1} \cdot K^{-1}$ at 38ºC) and diffusivity (α=1.53 10^{-7} $m^2 \cdot s^{-1}$ at 38ºC) of polycarbonate [24]. A temperature gradient appeared between the upper side of the DVD (surface with the reaction solution) and the bottom (hot surface), introducing a time delay before reaching the optimal amplification temperature on the analytical surface. The oven (non-contact heating device) provided a low evaporation percentage and high signals, and was selected for further experiments. Under these conditions, reproducible assays were also achieved (variation on the optical signal: 4-6%) and the equivalent amplification factor of the targeted DNA was $7.3 \cdot 10^8$-$8.9 \cdot 10^8$-fold, the equivalent to an average amplification efficiency of 1.11 per minute. This low *E*-value is common in solid-phase amplifications, as described above.

Proof of concept. Detection of *Salmonella*. Although the one-pot reaction can be performed by different sensors, the use of compact disc technology provides major advantages. Firstly, the mass production of discs and readers creates a high quality, low-cost, and high-access sensor. Secondly, polycarbonate substrates have excellent bioanalytical properties, such as high immobilisation yield of primers, minimal droplets displacement, and absence of non-specific backgrounds. Thirdly, high-working capability has been achieved due to the huge sensing area. Finally, the optical reader properties (laser drive), such as light weight (<500 g) or reduced volume (a few centimetres), are compatible with its implementation in different settings.

The analytical performance of the one-pot format based DVD technology was established and compared to those of the two-step approach. A triplex assay was tested for the generic detection of *Salmonella* spp. (genes *hns* and *oriC*) and for specific detection of *Salmonella* subspecies I (gene *STM4057*), and 432 independent spots per DVD (3 genes × 4 replicates × 36 zones) were printed. No primer-primer dimer, false positive or cross-contamination problems were observed.

A correlation between the amount of DNA extracted from pure cultures and the optical signal was obtained for the three genes up to a concentration of 200 ng/mL. As seen in Table 2, the lowest detectable concentration was 0.12-0.15 ng/mL, the equivalent to 24-30 copies/mL. The slightly worse results obtained for the heminested sp-RPA as compared to the two-step format (6-25 copies/mL) is explained by the poorer amplification efficiency of the solid-phase approach. The reproducibility of the integrated format, expressed as the relative standard deviation of three replicates, was

7.8-20.7%. These results were slightly higher than the two-step approach (5.9-16.3%), but were adequate for pathogen determination.

The reliability of the method for its application in large-scale screening was evaluated by the detection of *Salmonella* strains in food and clinical samples. The set was composed of raw samples and was spiked with several serotypes of *Salmonella* spp. and the non-target pathogen (*Cronobacter sakazakii*). Thirty-six samples/replicates were simultaneously analysed by a single assay to yield the results shown in Figure 4. The samples inoculated with *Cronobacter sakazakii* or non-inoculated food samples were negative for the three genes. Industrial sample (chicken carcass) and vaccines (human: *S. thyphi* and veterinary: *S. typhimorium* and *S. enteritidis*) were positive and showed a semi-quantitative correlation between the amount of pathogen and optical intensities, expressed as a signal/noise ratio for the microarray spots (Table 3). Hence an integrated amplification-detection of *Salmonella* spp. and subspecies I was achieved in a broad set of samples.

Numerous studies have been described for *Salmonella* detection. Conventional methods include microscopy, culture and serology, but they are time consuming and have worse sensitivity that our proposed approach [25]. Techniques based on PCR amplification are the most commonly used and show similar (or better) analytical performances but reduced portability [25-26]. In recent years, there has been much research activity in the area of biosensors, such as quartz crystal microbalance or latex agglutination assays, which have improved limits of detection and reduced time assay [25-27]. Recent biosensors have been published applying solid-phase RPA combined with silicon microring resonance, on-chip fluorescence, lateral flow strips or electrochemical detection [15, 16, 17]. These approaches have reported similar analytical performances, but have shown some drawbacks. For instance, the number of samples that can be detected simultaneously is smaller, time-consuming surface treatments are required and they need complex devices and expensive detectors. Conversely, no blocking/modification procedure was necessary for the polycarbonate surface of DVDs to the avoid inhibition of polymerases or the non-specific adsorption of amplification reagents. Also, in the DVD technology, the simultaneous analysis of 3 genes in 36 samples was allowed, and the DVD-drive is used as a low-cost, portable, sensitive and reproducible optical detector.

The analysis time of the solid-phase amplification was 40 min. The two-step strategy took approximately 88 min in all: in-tube amplification (40 min), hybridisation solution

preparation (~2 min), thermal denaturing (5 min), and the hybridisation process (40 min). Therefore, the one-pot strategy dramatically reduces sample handling and the assay time without compromising the results.

CONCLUSIONS

A simple method, an alternative to conventional tools (quantitative PCR or glass-slides microarray formats), has been developed to increase access to genomic information in non-specialised laboratories. The proposed system integrates DNA amplification and hybridisation in one process and on one platform. Moreover, the isothermal nature of the solid-phase RPA protocol simplifies the required heating system.

This is the first time that a solid-phase RPA approach in a microarray format is presented. The obtained data have shown that the system is a competitive, portable and robust sensor that integrates the amplification and hybridisation steps in a one-pot reaction to allow a multiplex analysis (e.g., number of genes and samples). It is worth mentioning that the proposed platform does not require microfluidic assemblies, which immensely simplifies the analytical process or the design/construction of the sensing platform. Furthermore, this method can be extended to other isothermal reactions (e.g., HDA), other detection approaches (e.g., membrane microarrays), or can be used to integrate microfluidic elements (e.g., reaction chambers).

The implementation of the one-pot method has been achieved for food safety control where multi-step approaches are time-consuming and prone to contamination. The reliable identification (inclusivity and exclusivity) of regulated infectious microorganisms is an important issue because disinfection techniques are target designed. It should be noted that the detection of *Salmonella* strains has been done by processing 36 samples of genomic DNA per disc in under 90 min. Despite the simplicity of the approach, the results demonstrate that this assay can be applied without compromising analytical performance and that it well suits routine genomic analysis (diseases diagnosis, bioterrorism, food safety and forensic areas).

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