Sensitive precise measurements were taken (high signal-to-noise ratios, 10-μm image resolution, 99% scan-to-scan reproducibility). These features confirmed their potential as analytical tools, are a competitive alternative to fluorescence scanners, and incorporate additional advantages, such as user-friendly interface and connectivity for telemedicine needs. The analytical performances of the integrated platform (assay and reader) in the human samples were also excellent, with a low detection limit (100 genomic DNA copies), and reproducible (<15%) and cheap assays (< 10 €/test). The correct genotyping of a genetic biomarker (single-nucleotide polymorphism located in the *GRIK4* gene) was achieved as the assigned genotypes agreed with those determined by using sequencing. The portability, favourable discriminating and read-out capabilities reveal that the implementation of mass-produced low-cost devices into minimal-specialised clinical laboratories is closer to becoming a reality.

Keywords

Single-nucleotide polymorphism; Isothermal DNA amplification; Point-of-care testing; Smartphone; Scanner; Compact disc.

INTRODUCTION

Advanced molecular technologies are a growing field in the healthcare system that address both diagnostics and treatment selection [1]. Current analytical methodologies enable measurements, basically in laboratories with specialised infrastructure and classical instruments (biochemical analysers, DNA sequencers, scanners, etc.). Alternative systems are needed to broaden the clinical facilities available to incorporate diagnostic tools. Point-of-care (POC) approaches offer fast, robust and reliable results and reduce medical costs, mislabelling and mishandling [2]. Thanks to these characteristics, POC tests are used for the prevention, control of disease outbreaks and monitoring health conditions, extending the medical scenarios to be addressed [3].

The recent advances made in materials, microfluidics and instrumentation have improved the performances of POC systems [4]. Particularly, the availability and affordability of consumer electronic, or home electronic, equipment, are increasing the possibilities of innovative solutions. They include devices used for entertainment, communications and home-office activities, such as smartphones, scanners, and compact disc drives. These hand-held optoelectronic devices have the potential to make biosensing more accessible to society [5]. Two categories can be defined depending on the employed sensing principle. The first is composed of digital imaging devices, which consists of an array of pixel sensors that converts light intensity into electrical current (charge coupled device or a complementary-metal-oxide-semiconductor). The optical sensors installed in smartphones [6], documental scanners [7], or similar devices, provide images of the assay platform, generally a planar or microfluidic chip, with enough quality to obtain analytical information [8]. In case of smartphones, custom-made attachments are used to hold the sample and auxiliary optical modules [9]. The second category is based on compact disc technology, where assays are performed on the optical disc surface and a disc drive acts as the optical scanner [10-13].

Consumer electronic equipment has been adapted as imaging platforms for genetic diagnoses with demonstrated sensitivity [14]. To reach the copy number required for molecular detection, a common challenge is the DNA amplification process (e.g. polymerase chain reaction, PCR). In POC approaches, one important component is the heat system for performing a precise fast thermal cycling [15]. In recent years, alternative methods have been explored using isothermal amplification techniques and simple heaters [16, 17]. Among the available options, the loop-mediated amplification method (LAMP) is the most popular solution [18]. This reaction amplifies DNA at constant temperatures by using the Bst polymerase large fragment, which presents great strand displacement activity and enzymatic processivity. LAMP also provides higher amplification yields than PCR in shorter incubation times.

Regarding applications, consumer electronic devices have been employed for detecting specific target (e.g. disease biomarker and infectious pathogen) [19-22]. However, several diagnostic and prognostic applications demand the simultaneous detection of multiple regions or variants (e.g. differentiation among similar pathogen strains or detection of point-mutations). In order to increase multiplexing capabilities, a common strategy is multiple parallel assays performed in microreactors on chips [23]. Another alternative is the combination with a hybridisation assay using probes immobilised on a chip surface, followed by adequate labelling and the optical detection of the corresponding array. In a recent paper, we demonstrated the potential of this approach combined to smartphone based-detection [24]. However, other consumer electronic devices are also potentially compatible to be used as readers of array-based assays. A scientific challenge for low-cost diagnostic community is an evaluation of their suitability and limitations, considering specific spectral responsivity, integration capabilities and associated data quality [25].

In this research, our goal was to explore the analytical capabilities of different consumer electronic techniques leverages for imaging of array chips of nucleic acids. The compared devices were smartphone, flatbed scanner, USB digital microscope and compact disc drive. Therefore, the study was to stablish the feasible requirements to transform each equipment into analytical reader of results generated by a highly sensitive and specific isothermal DNA assay. As proof of concept, the final POC integrated systems (biosensing assay and readers) were intended to be applied to the accurate low-cost discrimination of clinically relevant genetic variants.

MATERIAL AND METHODS

Primers and probes

The studied biomarker was the single nucleotide polymorphism (SNP) associated with the pharmacogenomics of anti-depressants drugs (rs1954787, g.285909T>C, located in the GRIK4 gene). LAMP primers and probes were selected according to the thermodynamic parameters associated with the perfect-match and mismatched duplexes (Supplementary Material). All the oligonucleotides used in this study were purchased from Eurofins (Luxembourg).

LAMP combined withallele selective hybridation

Genomic DNA amplification was carried out in 200-µL polypropylene vials. Each reaction (12.5 µL) was composed of 1 uisothermal amplification buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8), 1.5 M betaine, additional 6 mM $MgSO_4$, 1.2 mM dNTPs, 10 µM digoxigenin-11-deoxyuridine triphosphate (DIG-dUTP), 0.2 µM of outer primers, 1.2 µM of inner primers, 0.32 U/µL Bst polymerase 2.0

(New England Biolabs, USA) and 0.32 ng/µL of DNA. Vials were incubated at 62 qCfor 60 min (digital heat block, VWR, USA).

An allele-selective hybridisation assay led to the discrimination of the amplified products. Assays were performed on polycarbonate planar slides (detection device: chip-based sensors) or on the bottom layer of DVD discs (detection device: DVD drive). Probe arraying and the hybridisation of the amplification products were performed according to the protocol developed in previous work [26]. The array layout contained 4 replicates per probe and 10 arrays per chip. Regarding chip staining, the digoxigenin-labelled duplexes were recognised by horseradish-peroxidase-conjugated anti-digoxigenin antibodies, and were stained by deposition of 3,3',5,5'-tetramethylbenzidine (ep(HS)TMB, SDT reagents, Germany).

Array imagining

Four different consumer electronic technologies were examined to image the arrays according to the following protocols:

USBdigital microscopeThe profile intensities were measured by a portable microscope (Dino-Lite AM4013MZT, AnMo Electronics Co., Taiwan) based on a colour CMOS system (resolution 1.3 Megapixel, maximum frame rate: 30 fps). Images were captured by vertically positioning the equipment over the array at a 5-centimetre distance and employing the microscope internal LED as the light source (maximum illumination 18,500 lux). The DinoCapture 2.0 software was employed to record the image at the 1.3-megapixel resolution.

SmartphoneArray images were also captured by a smartphone (MotoG first generation, Motorola, EEUU) using a home-made chamber (8 x 6.7 x 4.4 cm) [24]. The device specifications were typical of a mid-range phone (display 4.5”, processor 1.2 GHz quad core, RAM 1 GB, rear camera 5-megapixel CMOS). The reading chamber had a frontal rectangular aperture for the smartphone camera, a lateral hole used to illuminate the array by an external

optical fibre light source (power 20W, LE.5209 model, Euromex, Holland), and an aperture for inserting the assay chip. After adjusting the focus and exposure (75% level), images were captured. The system did not require any connection to the telephone network.

Flatbed scanner. An office scanner (Perfection 1640SU Office, Epson, Japan), which incorporated a CCD image sensor, was also employed in the reflectance mode. The array support was positioned over the equipment bed and scanning was carried out at a 1,600 dpi (dots per inch) resolution using the Epson scan default software (disabled auto-correction functions).

Compact disc drive. The DVD-supported microarrays were directly read by a digital versatile disc (DVD) drive (LG DVD GSA-H42N, LG Electronics Inc., USA), which incorporated a data acquisition board model (DT9832A-02-OEM; Data Translation, Marlboro, MA, USA) [13]. The standard disc drive acted as a miniature high-precision optical device that consists of laser diodes, collimating lenses, diffraction gratings and a photodiode. The focus and tracking mechanism was responsible for spinning the disc and moving the optical pickup head unit. The reading conditions were adjusted by a custom software: rotation speed of 4× (13.46 m/s) and 21 dB gain at a detection rate of 1,700 mega-samples/s. Thus, the array image was formed from the data captured in each radius.

The ImageJ free-access software (National Institutes of Health, USA) was used to process the images in the tagged image file format (TIF) and on a 16-bit grey-scale (65,535 intensity values). The software provided the spot and surrounding background intensities. Signal-to-noise ratios were calculated as the net spot signal, divided by the background standard deviation.

Sample analysis

The performances of the POC systems for clinical routine were evaluated by applying SNP genotyping methods. Human subjects (n=15) were recruited for the present study according to ethics guidelines. Buccal smear samples were collected by a minimally invasive method. DNA extracts were obtained using a Purelink Genomic DNA mini kit (Thermo Fisher Scientific, USA). The genomic DNA content was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Extracts were diluted to 4 ng/µL (1,300 copies) and analysed as described in previous sections. The analysis was declared valid if the amplification and hybridisation controls provided a correct response. The genotype decision rule was constructed based on the signal-to-noise ratio associated with the wild-type (T) and mutant (C) probes.

Genotyping validation

Sanger sequencing was used to determine patient genotypes (blind samples). Each PCR reaction (12.5 µL) contained 1x amplification buffer, 3 mM $MgCl_2$, 200 µM dNTPs, 300 nM of forward and reverse primers, 0.5 units of Taq polymerase (Biotools, Spain) and 4 ng of DNA. The amplification was carried out in a thermal cycler (

Consumer electronics devices for DNA genotyping based on loop-mediated isothermal amplification and array hybridization

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ABSTRACT

Consumer electronic technologies offer practical performances to develop compact biosensing systems intended for the point-of-care testing of DNA biomarkers. Herein a discrimination method for detecting single nucleotide polymorphisms, based on isothermal amplification and on-chip hybridisation, was developed and integrated into user-friendly optical devices: e.g., USB digital microscope, flatbed scanner, smartphone and DVD drive. In order to adequately identify a single base change, loop-mediated isothermal amplification (LAMP) was employed, with high yields (8 orders) within 45 min. Subsequently, products were directly hybridised to the allele-specific probes attached to plastic chips in an array format. After colorimetric staining, four consumer electronic techniques were compared.