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Additional Information

Polymorphism genotyping based on loop-mediated isothermal amplification and smartphone detection

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ABSTRACT

The genotyping of a single-nucleotide polymorphism (SNP) is addressed through methods based on loop-mediated isothermal amplification (LAMP) combined with user-friendly optical read-outs to cover the current demand for point-of-care DNA biomarker detection. The modification of primer design and reaction composition improved the assay selectivity yielding allele-specific results and reducing false-positive frequency. Furthermore, the reduced cost, ease of use and effectiveness of colorimetric detection (solution and hybridization chip formats) were availed for the image capture by a smartphone, reching high sensitivity. In order to evaluate their discriminating capacities, LAMP-based methods were applied to human samples to genotype an SNP biomarker (rs1954787) located in the *GRIK4* gene and related to the treatment response to anti-depressants drugs. Sensitive (limit of detection: 100 genomic DNA copies), reproducible (<15% error), fast (around 70 min) and low-cost assays were accomplished. Patient subgroups were correctly discriminated, agreeing with reference sequencing techniques. The achieved analytical performances using the developed amplification-detection principles confirmed the approach potential for point-of-care optical DNA testing.

Keywords

Single-nucleotide polymorphism; loop-mediated isothermal amplification; point-of-care optical testing; smartphone; pharmacogenomics.

32 ## 1. Introduction

33 Rapid advances made in DNA biomarkers research are providing us with a better 34 understanding of disease mechanisms and drug action, which can lead to offering new 35 personalised medicine opportunities (Wooley et al., 2014). The key step for implementing such 36 systems in clinical routine is to employ highly efficient testing methods, which have to be 37 accurate and sensitive enough to detect even minority variants, but also practical and 38 economically feasible. In recent years, several studies have examined the capabilities of point-39 of-care (POC) genetic testing (Dobson et al., 2007). These tests generally include a cost-40 effective field-portable device, along with an accurate, sensitive and simple DNA assay.

41 Amplification reactions are central to DNA-based diagnostic methods because sensitivity 42 and selectivity depend on the effective increment in the copy number for the target region 43 (Vashist et al., 2015). The most widely used amplification method is polymerase chain reaction 44 (PCR), but it has some limitations for POC applications: a specific instrument for strict 45 temperature control, susceptibility to amplification yield variations related to reaction 46 conditions or the formation of air bubbles in miniaturised devices (Deng et al., 2015). Scientific 47 advances have led to several enzymatic reactions run at constant temperature that can be used 48 as an alternative to PCR-based amplification. Some recent reviews summarise isothermal 49 amplification reactions and their use as analytical tools (Craw et al., 2012; Yan et al., 2014; Li 50 2015).

51 Loop-mediated isothermal amplification (LAMP), developed by Notomi et al. 2000, is the 52 most extensively studied isothermal amplification technique. The main advantages over other 53 approaches are its high amplification yield, good tolerance to inhibitors, short time and 54 compatibility with several detection principles. The conventional approach relies on four 55 primers to recognise six different sequences of the target DNA, which also leads to very high 56 specificity. The action of a highly strand-displacing DNA polymerase (Bst polymerase) 57 generates large amounts of dumbbell-like structures under isothermal conditions (60–65°C). In 58 virtue of these features, LAMP-based methods have been extensively applied to diagnose 59 infectious diseases by detecting bacteria, viruses and parasites (Parida et al., 2008; Connelly et 60 al., 2015).

61 In the last few years, several studies have demonstrated LAMP's capability to discriminate 62 single-base variations, such as single nucleotide polymorphisms (SNPs) and somatic point 63 detection. These methods are based on allele-specific hybridisation (Jiang et al., 2015, 64 Nakamura et al., 2007), and amplification using allele-specific primers (Zhang et al., 2014; 65 Yongkiettrakul et al., 2017) or a blocking agent (Itonaga et al., 2016). However, these methods

66 usually rely on naked-eye visualisation or carry out the detection with expensive and bulky
67 laboratory equipment (e.g. electrochemical stations, real-time turbidimeter or fluorometer).
68 With the adequate integration to user-friendly detection technologies, these LAMP variants are
69 appealing to develop POC testing. Examples of candidate clinical challenges are to select the
70 correct oncological treatment with monoclonal antibodies (Shackelford et al., 2012), and to
71 adjust drug doses in neuropathies and psychiatric disorders (Chan et al., 2011, Hamilton, 2015).
72 We herein explored these discrimination principles to develop high-performance POC
73 systems. The first method involved the allele-specific oligonucleotide hybridisation of the
74 LAMP products in the stem-loop region (LAMP-ASO). The second was based on the annealing
75 selectivity of allele-specific inner primers (3'AS-LAMP), while discrimination in the third
76 approach relied on DNA synthesis from a dumbbell-like starting structure (5' AS-LAMP). The
77 key conditions to obtain adequate amplification yield, improve the discrimination factor and
78 reduce false-positive frequency, were investigated. To this end, modifications in the
79 primer/probe design, and variations in the amplification or hybridisation mix composition,
80 were included.
81 Detection of allele specific products in POC scenarios also requires alternative detectors to
82 previous LAMP approaches. In line with this, the features of consumer electronic devices are
83 excellent as they are ubiquitous, low-cost, compact and high-performance products that can
84 benefit advanced analytical measurements (Kido et al., 2000; Maquieira, 2012; Ozcan, 2014;
85 Quesada-González et al., 2016). The sensing devices described for diagnostic purposes include
86 compact disc drivers (Morais et al., 2014), flatbed scanners (Tortajada-Genaro et al., 2016) and
87 mobile phones (Roda et al., 2016; Kanchi et al., 2018), among others. In this study, we explored
88 the colorimetric detection of the developed homogeneous and heterogeneous LAMP assays
89 supported by smartphone technology due to its widespread presence, portability and capacity
90 to transmit data at a user-friendly interface. This integrated system also fulfils WHO
91 requirements, and corresponds to the acronym "ASSURED": affordable, sensitive, specific,
92 user-friendly, rapid and robust, equipment-free, and delivered to those who need it.
93
94 **2. Material and Methods**
95 *2.1. Primers and probes*
96 LAMP primers and probes were designed for the target SNP according to the
97 thermodynamic parameters described in the literature (Notomi et al., 2000, Tortajada-Genaro
98 et al., 2017). The complete design strategy and oligonucleotide sequences can be found in the

99 Supplementary Material. All the oligonucleotides used in this study were purchased from
100 Eurofins (Luxembourg).

101 *2.2. LAMP combined with allele-selective oligonucleotide hybridisation: LAMP-ASO*
102 *method*

103 In this approach, isothermal amplification was followed by hybridisation to the specific
104 probes immobilised on planar polycarbonate chips (25 × 75 mm). Non-allele selective LAMP
105 amplification was carried out in 200 µL propylene phials with primers that enclosed the
106 polymorphic site. Each reaction (12.5 µL) was composed of 1× isothermal amplification buffer
107 (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8),
108 1.5 M betaine, further 6 mM $MgSO_4$, 1.2 mM dNTPs, 10 µM digoxigenin-11-deoxyuridine
109 triphosphate (DIG-dUTP), 0.2 µM of outer primers, 1.2 µM of inner primers, 0.32 U/µL Bst
110 polymerase 2.0 (New England Biolabs, USA) and 0.32 ng/µL (approximately 100 copies per
111 µL) of the studied DNA. Vials were incubated at 62°C for 60 min (digital heat block, VWR).
112 Amplification products were then hybridised with the allele-specific oligonucleotide probes
113 immobilised on chips in a microarray format. Probe arraying, hybridisation and colorimetric
114 staining were performed according to the protocol developed in previous works (Tortajada-
115 Genaro et al., 2016, Yamanaka et al., 2017). The resulting hybridisation products anchored to
116 the surface were recognised by horseradish peroxidase-conjugated antibodies and stained by
117 3,3',5,5'-tetramethylbenzidine to produce a precipitate.

118 A digital imaging technique was used to record the LAMP-ASO results. Array images
119 were captured by a smartphone (MotoG first generation, Motorola) using a homemade capture
120 chamber (8.0 x 6.7 x 4.4 cm). This chamber had a frontal rectangular aperture for the
121 smartphone camera, a lateral hole to illuminate the array by an external optical fibre light source
122 (20W power, 3,000 K colour temperature, LE.5209 model, Euromex, Holland), and an inferior
123 aperture to insert the assay chip. The image was captured after adjusting both focus and
124 exposure (75% saturation) and was converted into a tagged image file format on a 16-bit (0-
125 65,535) greyscale with the ImageJ software (National Institutes of Health, USA). Images were
126 analysed and the resulting spot intensities were expressed in signal-to-noise ratio terms.
127

128 *2.3. Allele-specific LAMP: 3'AS-LAMP and 5' AS-LAMP formats*

129 Two homogeneous amplification formats were assayed using allele-specific primers (see
130 Supplementary Material). In each case, discrimination was achieved using two reaction
131 mixtures to amplify the wild-type variant (wild-type primers) or the mutant variant (mutant

132 primers). For 3' AS-LAMP format, the polymorphism was located at the 3'-end of the forward
133 inner primer (FIP), leading two allele-specific primers and a reverse inner primer common to
134 both reaction mixtures. Therefore, the reaction mixture composition varied from the previously
135 described non-selective LAMP by using each FIP primer, 1.25 M betaine and 300 µM
136 hydroxynaphtol blue. In the 5' AS-LAMP format, the polymorphism was located at the 5'-end
137 of both FIP and BIP, and the difference in mixture composition was the betaine and dyer
138 concentrations, which were 0.75 M and 300 µM, respectively. On-chip amplification was
139 carried out with a rhombic chamber chip (reaction volume 10 µL, Zeonor material) supplied
140 by microfluidic ChipShop (Germany). Inlets and outlets were connected directly to Tygon
141 tubing. Chips were loaded with the amplification samples and were incubated at 62°C for 60
142 min.
143 Smartphone imaging enabled end-point direct colorimetric detection. For this purpose,
144 the reaction chip with a reference colour palette was placed in the previously described
145 detection assembly. The AssayColor software (Alidans, Italy), installed in the smartphone, was
146 used to capture and analyse images. This scientific application, developed for the Android
147 operating system, provided colour intensities in the red, green and blue channels (RGB) for
148 each LAMP product. The R/G intensity ratio was selected as an analytical signal.
149
150 *2.4. Sample analysis*
151 Subjects (n=15) were recruited according to ethics with informed consents. DNA extracts
152 were obtained from the buccal smear samples with the Purelink Genomic DNA mini kit
153 (Thermo Fisher Scientific, USA). Purified products were eluted with Tris-HCl buffer (Tris 10
154 mM, pH 8.6) and their genomic DNA content was quantified in a NanoDrop 2000
155 spectrophotometer (Thermo Fisher Scientific, USA). A 260/280 nm absorbance ratio above 1.8
156 was considered to determine adequate purity. Extracts were diluted to 4 ng/µL and stored at -
157 20°C until further use. Subsequently, samples were submitted to the LAMP-ASO, 3'AS-LAMP
158 and 5'AS-LAMP methods. A no-template control and a *Salmonella typhimurium* DNA extract
159 were used to check for false-positive assays. A discrimination index was calculated from the
160 signal of the wild-type (WT) and mutant (MUT) responses according to the following equation:
161 (WT − MUT)/(WT + MUT). The genotype was assigned according to discrimination
162 thresholds (TT higher than +0.33, TC between +0.33 and -0.33, and CC lower than -0.33).
163

164 *2.5. Genotyping validation*

165 Two techniques were used to confirm patients' genotypes: Sanger sequencing and allele-
166 specific PCR.

167 For Sanger sequencing, each PCR reaction was carried out in a mixture (12.5 µL) that
168 contained 1x amplification buffer, 3 mM $MgCl_2$, 200 µM dNTPs, 300 nM of the forward and
169 reverse primers, 0.5 units of Taq polymerase (Biotools, Spain) and 20 ng of genomic DNA per
170 reaction. Amplification was carried out in a UnoCycler thermal cycler (VWR, USA) according
171 to the following programme: initial denaturation at 95°C for 5 min, followed by 35
172 amplification cycles of denaturation (95°C for 30 s), annealing (60°C for 30 s) and elongation
173 (72°C for 30 s), and a final extension step at 72°C for 5 min. The resulting amplification
174 products were diluted, extended with fluorescent dideoxynucleotides (Big Dye Terminator
175 Cycle Sequencing Kit v3.1, Thermo Fisher Scientific, USA), and analysed in a fluorescence-
176 capillary sequencer (ABI 3130xl Genetic Analyzer, Applied Biosystems, USA).

177 Allele-specific PCR (AS-PCR) was based on the use of two forward primers that differed
178 at the 3'-end nucleotide, and were complementary to the wild-type or mutant variant. An
179 additional mismatch at the penultimate nucleotide was included. The amplification conditions
180 were identical to those previously described for PCR, except for the use of the allele-specific
181 primers and an annealing temperature of 62°C. End-point fluorescence was measured to
182 confirm amplification. Products were diluted in 0.5× SYBR Safe (Invitrogen, USA) on a 96-
183 well black polystyrene plate and analysed in a plate reader (Victor 3TM V1420, Perking Elmer,
184 Finland) at excitation and emission wavelengths of 485 and 535 nm, respectively.

185

186 The Statgraphics Centurion statistical package for Windows v.16 was used for the data
187 analysis.

188 **3. Results and Discussion**

189 *3.1. ASO-LAMP set-up*

190 SNP discrimination was performed with the combination of isothermal DNA
191 amplification and hybridisation with allele-selective probes in a solid-phase format.

192 The first step was the oligonucleotide design (primers and probes). There were two
193 design options according to the target polymorphism location in the LAMP product loop-
194 structure: central position (double-strand region) or loops (single-strand regions). The second
195 option was chosen to improve the hybridisation yield to the array probe (Fig. 1a). A
196 thermodynamic analysis was used to select the candidate probes that maximised the
197 hybridisation of perfect-match pairs (wild-type or mutant) and hampered the coupling of
198 mismatched products (wild-type product/mutant probe or mutant product/wild-type probe). An
199 additional design restriction was the central position of the polymorphic mismatch in the probe
200 to increase assay selectivity. The selected sequences produced wide variation in standard free
201 energies, expressed as the difference between the single-base mismatch (ΔG^{o}_{mutant}) and the
202 perfect match ($\Delta G^{o}_{wild\text{-}type}$). Estimated values were 3.3-4.5 kcal/mol.

203 The LAMP reaction was optimised to selectively amplify the targeted region using the
204 designed non-allele-specific primers. Reagent concentrations (enzyme, inner primers and outer
205 primers), amplification temperature and reaction time were studied by the fluorescence analysis
206 (see the Supplementary Material). Negative controls (non-human DNA) produced a signal
207 comparable to the background, while the amplification of the human DNA template generated
208 a significantly distinguishable signal (Fig. 1b). The wild-type and mutant templates produced
209 similar amplification curves, and the time selected for the end-point analysis was 60 min. The
210 amplification factor was $(2.6\pm0.8)\times10^{8}$, which gave a 23-fold higher yield than a typical PCR
211 using the same external primers and the amount of the initial DNA template.

212 The next experiments focused on the selective hybridisation to the probes anchored to
213 the plastic chip, by directly dispensing the end-point LAMP product on the probe arrays. This
214 approach is simpler and more efficient than combining PCR-based methods with microarray
215 detection because an intermediate (thermal or chemical) denaturalisation step is generally
216 required (Wooley et al., 2014, Tortajada-Genaro et al., 2016). The probe immobilisation
217 parameters (concentration, drop volume, and surface treatment) and the hybridisation variables
218 (buffer composition, time and washing cycle) were studied to balance yield and selectivity, as
219 described in the Supplementary Material. The most critical variable to achieve selective
220 hybridisation was buffer composition, particularly formamide concentration (Figure 1c). Under

221 the optimal conditions (1x sodium saline citrate buffer, 30% formamide), a detectable signal
222 was obtained for the perfect-matched duplexes (wild-type and mutant homoduplex), while a
223 background-equivalent response was acquired for the mismatch hybrids.
224

225 *3.2. AS-LAMP set-up*

226 In the preliminary studies, a non-specific amplification signal was generated for the non-
227 matched primer-template pairs. The LAMP assays generated false-positives due to the
228 formation of unexpected primer structures, as observed in other studies (Connelly et al., 2015;
229 Wang et al., 2015). Therefore, several modifications were made to increase assay selectivity in
230 the AS-LAMP formats. Firstly, an additional mismatch was deliberately added to the
231 penultimate nucleotide of the allele-specific primers. Major destabilisation of the hybridization
232 process was estimated for the mismatch probes, where the calculated variation of the standard
233 free energies was about 1.2 - 2.8 kcal/mol. Secondly, the effect adding betaine to the
234 amplification mix was evaluated. This amino acid analogue is often used for destabilising
235 dsDNA and for reducing the sequence composition influence on the melting temperature. The
236 experiments showed that adding betaine eliminated the false-positive results associated with
237 the mismatch hybrids (Fig. 2). However, increasing the betaine concentrations also led to the
238 undesired inhibition of the perfect-matched duplexes. The inhibition effect was more
239 prominent in the 5' allele-specific format than in the 3' one. This could be explained by the
240 lesser stability of the associated perfect-match hybrids (about 5 kcal/mol) and a different
241 number of allele-specific primers (two in the 5' format and one in the 3' format). In summary,
242 the results at the selected values (1.25 M for 3'AS-LAMP and 0.75 M for 5'AS-LAMP) showed
243 better amplification selectivity compared to conventional conditions.
244 Amplification kinetics was studied to verify the discrimination capacity and the assay
245 turnout time for the LAMP reactions. Both the allele-specific methods showed adequate
246 selectivity as the real-time signals for the no-template control and the non-human DNA extract
247 (*Salmonella* culture) were similar to the background. When the perfectly matched primers were
248 used, amplification started at 40 min for the 3' and 5' allele-specific approaches, while the
249 mismatched primers generated a signal after a delay that went beyond 30 min in both cases. It
250 is worth noting that the stability difference between the previously described matched and
251 mismatched duplexes was also reflected in the amplification kinetic profiles. Longer delays for
252 the mismatched pairs were found in the 3' format. After considering the results, a 60-minute
253 amplification time was selected for the following experiments to prevent the formation of non-
254 specific products during the assay.

3.3. Smartphone detection

The detection of the previously described allele-selective products was achieved with conventional laboratory instruments; i.e. fluorescence qPCR thermocycler, fluorescence spectrophotometer or fluorescence scanner. The next challenge was to adapt the methods for colorimetric detection using a smartphone (complementary metal–oxide–semiconductor, CMOS sensor) suitable for point-of-care testing. An integrated detection device was assembled for chip reading, which comprised a light source, a dark chamber and the smartphone aligned to the chip (Fig. 3). To guarantee inter-assay measurement robustness, a colour pattern (a violet to blue scale) was photographed together with the assay platforms. The specific measuring conditions were optimised to digitalise the array profile by the smartphone camera, as the Supplementary Material describes. Image resolution, expressed as pixel width, was 17 μm.

For the LAMP-ASO approach, a colorimetric detection method for the probe-LAMP product hybrids based on an immunorecognition step (digoxigenin/primary antibody/secondary antibody system) and enzymatic staining (horseradish peroxidase/colorimetric substrate system) was studied. If hybridisation was positive, a blue precipitate was generated on the spot by attenuating the captured optical density (reflection-mode detection). The intensity of each array spot (400 μm diameter) was calculated as the average of 448 pixels. A perfect-match interaction (LAMP product/probe) produced signals up to 56,000 a.u. in 16-bit greyscale units, while the chip background values were in the range of 7,000±400 a.u.. Therefore, the spot intensities discriminated positive and negative recognition events depending on the probe/product pair. Statistical significance was calculated by a Student's *t*-test, and p-values were <0.05 in all cases. This study demonstrates, for the first time, the colorimetric detection of allele-specific hybridisation LAMP products, which produces excellent versatility and is a key factor to make a simpler reader-suitable method for POC applications.

For the AS-LAMP approaches, the addition of a magnesium indicator (hydroxynaphtol blue) was evaluated (Zhang et al., 2014b). Along with the capacity of the isothermally amplifying double strand DNA, a very high yield is an interesting advantage that LAMP offers over conventional PCR and other isothermal amplification methods, as it allows a subsequent direct colorimetric detection with a smartphone. This staining method was simple and did not require any additional devices (i.e. ultraviolet source, wavelength filters, magnification lens). Detection was achieved with no post-amplification steps. To improve the recorded responses

288 (scattered light), the concentration of hydroxynaphtol blue was gradually increased and the
289 light intensity for the RGB channels was recorded (Fig. 3). Concentrations above 300 µM
290 provided a significant signal of red channel for the positive amplifications compared to the
291 negative controls (test t: $t=2.25$, $p<0.05$). From the obtained results, the proposed modification
292 of ASA-LAMP approaches showed excellent signal discrimination, which indicates its
293 potential as a polymorphism biomarker analysis tool.
294
295 *2.4. Comparing methods*
296 The main features and analytical performances of the three methods were subsequently
297 compared (Table 1). Analytical sensitivity and reproducibility were calculated from the
298 consecutive dilutions of a genomic human DNA template. Although naked-eye colour
299 observation was possible to visualise positive amplification (violet to sky blue), the use of an
300 imaging/sensing device guaranteed reliable measurements when smaller amounts of the target
301 SNP were present in the sample and colour change was subtle. The estimated limit of detection
302 was 100 copies for the all LAMP-smartphones-based methods. Thus the required amount of
303 genomic DNA was smaller than previous LAMP approaches (Nakamura et al., 2007, Itonaga
304 et al., 2016), some genotyping assays (Gibriel et al., 2017) and sequencing techniques
305 (Goodwin et al., 2016). Assay repeatability, calculated from replicates, yielded error rates
306 under 15% in all the formats, which were lower than those obtained by naked eye visualisation
307 and similar to other SNP methods that have been applied to human samples.
308 The technical requirements for developing point-of-care systems were also evaluated.
309 The estimated reagent cost of LAMP-ASO was 2.65-fold higher than the AS variants, mainly
310 because of immunoreagent prices. Compared with the corresponding PCR approaches, LAMP
311 assays were more expensive (about 1.5-fold), mainly due to the cost of enzymes (Bst
312 polymerase *versus* Taq polymerase). In contrast, the LAMP approaches only required a low-
313 cost heating system (62°C; i.e. heater) compared to the conventional thermal cycler used in
314 PCR-based methods, along with a cheaper and more practical detector. The LAMP methods
315 also worked in shorter analysis times than their equivalent PCR approaches. The AS-LAMP
316 formats were the quickest (70 min) compared to LAMP-ASO (140 min), AS-PCR (120 min)
317 or PCR-ASO (190 min), mostly because of the shorter amplification times in the LAMP-based
318 methods. Hence these results are similar, or better, than those obtained for previous LAMP
319 approaches (Connelly et al., 2015; Safavieh et al., 2016; Itonaga et al., 2016).
320

321 *2.5. Patient sample analysis*

322 Psychiatric pharmacogenetics is a candidate field for developed POC genotyping
323 methods (Milanesi et al., 2015). As proof of concept, the genotyping of the rs1954787
324 polymorphism, located in the *GRIK4* gene, was selected to determine the genetic predisposition
325 of antidepressant treatment from the human DNA (n=15) extracted from buccal swabs. Only
326 by following the developed methodology were signals sufficiently different to achieve a
327 specific response profile depending on the genetic variant. Figure 4 shows the subsequent
328 discrimination graph. The three methods provided the same genotypes for all patients, except
329 for patient 8 in the LAMP-ASO approach. Nevertheless, the homogeneous approaches (3' AS-
330 LAMP and 5' AS-LAMP methods) provided clearer discrimination factors than the solid
331 hybridisation format (LAMP-ASO) due to their lower signals for the mismatched reaction
332 mixtures. Among the analysed samples, six patients (40%) were identified as being mutant
333 homozygous (CC) which can be related to a better chance of positive responses to depression
334 treatment (Horstmann, 2010). There were also six heterozygous patients (40%), who were
335 expected to give a normal response for drugs like citalopram. Finally, the results indicated that
336 three (20%) subjects presented a homozygous wild-type genotype (TT), which indicates a
337 higher risk of a non-response. Another comparison of the reference results (Sanger sequencing
338 and AS-PCR) revealed a perfect correlation with the genotypes determined by the LAMP-
339 based assays.

340 The clinical implications of this *in vitro* diagnostic assay were analysed. Major
341 depressive disorder affects were about 10-15% of the population (annually), with a degree of
342 uncertainty about the individual efficacy of the antidepressant treatment (Kawaguchi et al.,
343 2014). The discrimination of specific polymorphisms can enable quick personalised patient
344 management with a strong effect on therapy. Clinical trials have identified an association of
345 rs1954787 with therapy effectiveness, and have reported that CC homozygotes are more likely
346 to respond to treatment than TT homozygotes. Therefore, a simple low-cost genotyping tool
347 can support the better dosing of antidepressants.

348

349 **4. Conclusion**

350 This research confirms the excellent features of LAMP as a viable alternative to current
351 methodologies whose aim is genotyping purposes in order to overcome the associated technical
352 barriers. This study particularly supports the oligonucleotide design and the selection of
353 reaction conditions for colorimetric detection in both homogeneous and heterogeneous
354 formats. An accurate control of these experimental variables is required because false-positive

355 results are more frequent than for PCR-based methods. Our results endorse the technical
356 capabilities of smartphones as analytical readers for molecular diagnostic systems. Despite
357 having a worse optical resolution than benchtop instruments, CMOS sensor chips incorporated
358 into phone cameras offer adequate imaging features and widespread availability, which make
359 them ideal detectors for cost-effective assays. Compared to other electronic devices,
360 smartphone technology has additional advantages, such as assay reader, given its capability to
361 transmit data, ubiquity and users' familiarity to handle it.

362 The achieved LAMP discrimination process and low-cost detector combination shows
363 excellent performance and a wide dynamic range, which allows the technique to be
364 extrapolated other target genetic biomarkers. This offers researchers the chance to develop
365 integrated systems, which enable quicker monitoring of genetic predispositions to develop
366 certain diseases or to predict genomic-related responses to drug therapies.

367

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372

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