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1 **Development and comparison of a novel multiple cross displacement amplification**
2 **(MCDA) assay with other nucleic acid amplification methods for SARS-CoV-2 detection**
3

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18 ## Abstract

19 The development of alternative isothermal amplification assays including multiple cross
20 displacement amplification (MCDA) may address speed and portability limitations of real-
21 time PCR (rt-PCR) methods for SARS-CoV-2 detection. We developed a novel SARS-CoV-
22 2 MCDA assay and compared its speed and sensitivity to loop-mediated isothermal
23 amplification (LAMP) and rt-PCR. Two MCDA assays targeting SARS-CoV-2 N gene and
24 ORF1ab was designed. The fastest time to detection and sensitivity of MCDA was compared
25 to LAMP and rt-PCR using DNA standards and transcribed RNA. For N gene, MCDA was
26 faster than LAMP and rt-PCR by 10 and 20 minutes, respectively with fastest time to
27 detection at 5.2 minutes. rt-PCR had highest sensitivity with limit of detection at 10 copies/µl
28 compared with MCDA (100 copies/µl) and LAMP (500 copies/µl). For ORF1ab, MCDA and
29 LAMP had similar speed with fastest time to detection at 9.7 and 8.4 minutes, respectively.
30 LAMP was more sensitive for ORF1ab detection with 50 copies/µl compared to MCDA (500
31 copies/µl). In conclusion, different nucleic acid amplification methods provide different
32 advantages. MCDA is the fastest nucleic acid amplification method for SARS-CoV-2 while
33 rt-PCR is the most sensitive. These advantages should be considered when determining the
34 most suitable nucleic acid amplification methods for different applications.

35

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36 # Introduction

37 Rapid, portable and highly sensitive assays are essential to controlling the COVID-19
38 pandemic. Real-time-PCR (rt-PCR) is the gold standard for detection of SARS-CoV-2
39 genetic material[1]. However, rt-PCR requires trained personnel, advanced equipment and
40 relatively long assay times making it unsuitable for large-scale community screening. Other
41 tests developed include serological assays that rely on IgM/IgG antibodies which takes ~5
42 days to appear after symptom onset making them unsuitable for rapid early detection[2].

43 The development of alternative nucleic acid amplification methods including loop-mediated
44 isothermal amplification (LAMP) may offer improved speed, sensitivity and portability for
45 SARS-CoV-2 detection[3]. Another isothermal nucleic acid amplification method, called
46 multiple cross displacement amplification (MCDA) which uses 10 primers instead of six, has
47 also been suggested to have even higher sensitivity and speed than LAMP but has not yet
48 been developed for SARS-CoV-2 detection[4,5].

49 Despite claims of increased speed and sensitivity from isothermal amplification methods, no
50 study has directly compared the speed and sensitivity of these three different nucleic acid
51 amplification methods. Hence, here we developed an MCDA assay for SARS-CoV-2
52 detection and compared its speed and sensitivity to existing LAMP and rt-PCR methods.

53

54 # Methods

55 ## MCDA target gene selection

56 To identify target genes with highly conserved regions and a suitable GC-content for MCDA,
57 1,216 SARS-CoV-2 genomes deposited in GISAID (all available complete, high coverage
58 genomes (>29,000 bp) with low coverage flags excluded up until March 26, 2020)[6] were
59 aligned against the SARS-CoV-2 reference genome: NC_045512.2 using Snippy (v4.3.6)

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60 with the -ctgs flag and default settings (https://github.com/tseemann/snippy). A sliding
61 window approach was then applied to identify conserved 300bp windows with GC content
62 >43%, low SNP number, and low total SNP number (total SNPs was defined as the number
63 of strains with a SNP in a given window). Three 300 bp conserved regions were identified
64 and selected for MCDA primer design with two regions in ORF1ab (NC_045512.2: 515-831
65 and 12968-13288) and one in the *N* gene (NC_045512.2: 28345-28647).
66

67 MCDA primer design

68 For each region, 4 sets of MCDA primers were designed as previously described[5]. Each
69 primer set consisted of 2 cross-primers (CP1/CP2), 2 displacement primers (F1/F2) and 6
70 amplification primers (C1/C2, D1/D2, R1/R2) (Supplementary Table 1). Non-specific primer
71 binding was assessed using BLASTN against 14 non-SARS-CoV-2 coronaviruses used in
72 Lamb *et al.*[7], human genome (hg19) and 11 other common bacterial and viral respiratory
73 pathogen/microbiome species.
74

75 Preparation of DNA/RNA standards

76 For each region, ~500 bp gene fragments for ORF1ab (NC_045512.2: 416-931 and 12869-
77 13388) and N gene (NC_045512.2: 28246-28747) were synthesised with an additional 100 bp
78 up and downstream of the target region (ThermoFisher) (Supplementary Table 2). Each
79 fragment contained a T7 promoter for transcription and M13 adapters for amplification.
80 Seven DNA standards from synthesised gene fragments were prepared for ORF1ab (100,000,
81 10,000, 5000, 1000, 500, 250 and 100 copies/µl) while eight DNA standards were prepared
82 for the N gene (100,000, 10,000, 5000, 1000, 500, 250, 100 and 10 copies/µl).

83 For RNA, 1 pg of synthesised gene fragments were transcribed overnight at 37°C using T7
84 RNA polymerase (Sigma). Overnight DNA digestion was performed using the turbo DNA

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85 free kit (ThermoFisher) and further treated with DNase I (NEB) until all traces of DNA were
86 removed. Complete DNA removal was confirmed after each round of DNase treatment using
87 rt-PCR with the SensiFAST SYBR kit (Bioline) and F1/R1 MCDA primers (Supplementary
88 Table 1). The transcribed RNA was serially diluted and used as input. Since the amount of
89 RNA transcribed was below the 250 pg/µl limit of detection for qubit HS RNA assay
90 (ThermoFisher), the input RNA copy number could not be determined. Therefore, the lowest
91 detectable dilutions were used for sensitivity comparison.
92

93 **Initial evaluation of MCDA primer sets and optimisation of isothermal amplification**
94 **temperature**

95 MCDA reactions were performed using the WarmStart LAMP (DNA and RNA) kit (NEB)
96 which contains a warmstart RTx reverse transcriptase and Bst2.0 polymerase for
97 simultaneous reverse transcription and isothermal amplification. Antarctic thermolabile UDG
98 was also added in each reaction to prevent carryover contamination.
99

100 For each primer set, a primer mix containing: 3.3 µM of F1 and F2, 6.67 µM of C1 and C2,
101 10 µM of R1, R2, D1 and D2 and 20 µM of CP1 and CP2 was used. Standard desalting
102 purified primers were used for the initial evaluation and optimisation tests while HPLC
103 purified primers were used for sensitivity and speed comparison against LAMP and rt-PCR
104 tests.
105

106 For the initial evaluation of each MCDA primer set, a 10 µl reaction was used and contained:
107 5 µl of 2x WarmStart master mix, 0.2 µl of fluorescent dye, 1.2 µl of MCDA primer mix, 0.2
108 µl of 1U/µl Antarctic thermolabile UDG (NEB), 0.7 µl of 10 mM dUTP, 1.7 µl of H_2O and 1
109 µl of 1000 copy/µl DNA template (final reaction concentration = 100 DNA copies/µl). The

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110 final concentration of each MCDA primer in the reaction was 0.4 μM of F1 and F2, 0.8 μM
111 of C1 and C2, 1.2 μM of R1, R2, D1 and D2 and 2.4 μM of CP1 and CP2. Specificity of
112 MCDA primer sets were also evaluated using purified human genomic DNA (Sigma) and a
113 microbial community DNA standard (Zymo Research). MCDA reactions were performed in
114 triplicates in the Rotor-Gene Q (Qiagen) with isothermal amplification at either 60°C, 63°C or
115 65°C for 1 h and real time fluorescence detection every 60 seconds, followed by enzyme
116 inactivation at 95°C for 5 min and a final melt curve from 50°C – 99°C to ensure correct
117 MCDA product.
118

119 **Comparison of MCDA, LAMP and rt-PCR**

120 To compare the speed and sensitivity (limit of detection) of MCDA, LAMP and rt-PCR,
121 published primers targeting the same SARS-CoV-2 MCDA ORF1ab (NC_045512.2: 416-
122 931) and N (NC_045512.2: 28246-28747) regions were used (Supplementary Table 1). For
123 LAMP, two published primer sets from Zhang et al.[3] which targeted the same region as our
124 MCDA were compared. For rt-PCR, there were no suitable published primers pairs which
125 targeted the same ORF1ab region, therefore only primers submitted by the National Institute
126 of Health, Thailand against the N gene was compared[8]. All primers were HPLC-purified
127 grade.
128

129 MCDA, LAMP and rt-PCR were tested in three independent runs (biological replicates)
130 using the same aliquot of DNA/RNA. Each run contained 3 technical replicates. The limit of
131 detection was defined as the highest dilution where all 9 replicates (3 biological replicates x 3
132 technical replicates) were detected.
133

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134 To reduce between run variations, 10 µl MCDA and LAMP reactions were set up and
135 performed simultaneously in the same run. MCDA reactions were prepared as described
136 above. For LAMP, each 10 µl reaction contained: 5 µl 2x WarmStart master mix (NEB), 0.2
137 µl 50x fluorescent dye (NEB), 0.2 µl 1U/µl Antarctic thermolabile UDG (NEB), 0.7 µl 10
138 mM dUTP, 1 µl LAMP primer mix, 1.9 µl of H_2O and 1 µl of DNA/RNA template. Each
139 LAMP primer mix contained 16 µM FIP and BIP, 2 µM F3 and B3 and 4 µM LF and LB.
140 The final concentration of each LAMP primer in the reaction was 1.6 µM FIP and BIP, 0.2
141 µM F3 and B3 and 0.4 µM LF and LB. MCDA and LAMP isothermal amplification was
142 performed at 65°C as described above. The normalised fluorescence threshold line for N gene
143 amplification was set above the background fluorescence at 0.2 for MCDA and LAMP. For
144 ORF1ab MCDA and LAMP, the normalised fluorescence threshold line was set at 0.4 as
145 background fluorescence was higher. The detection time for MCDA and LAMP was defined
146 as the time it takes for the fluorescence intensity to pass the threshold line.
147
148 For rt-PCR using DNA templates, 10 µl reactions containing 5 µl SensiFAST probe No-ROX
149 mix (Bioline), 0.5 µl rt-PCR primer mix (40 µM F and R, 10 µM probe), 3.5 µl of H_2O and 1
150 µl DNA template were used. The final concentration of each rt-PCR primer and probe in the
151 reaction was 2 µM F and R and 0.5 µM probe. The cycling conditions were 95°C for 2 min,
152 followed by 45 cycles of 95°C for 15 secs and 55°C for 30 secs.
153
154 For rt-PCR with RNA templates, 10 µl reactions were set up containing 5 µl SensiFAST
155 probe No-ROX One-Step mix (Bioline), 0.5 µl primer mix (40 µM F and R, 10 µM probe),
156 0.1 µl reverse transcriptase (Bioline), 3.4 µl of H_2O and 1 µl RNA template. Reverse
157 transcription was performed at 45°C for 20 minutes followed by rt-PCR amplification as
158 described above for DNA.

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159

160 To compare the speed of rt-PCR, cycle threshold (Ct) was converted to time using the
161 following equation: Time = (Ct x 50 sec) + 120 sec. The detection time required for rt-PCR
162 was calculated based on the cycling conditions (45 sec per cycle plus an initial 120 sec hold)
163 and the ramp rate for the Rotor-gene Q (5 sec per cycle). The ramp rate for the Rotor gene Q
164 is 15°C/s for heating and 20°C/s for cooling according to the manufacture's technical
165 information (https://www.qiagen.com/us/resources/download.aspx?id=2120af5e-8daf-4184-
166 b277-aeb6ef5bbc05&lang=it-IT).

167

168 ## Results

169 ### Development of MCDA assays for SARS-CoV-2 detection

170 Three 300 bp conserved regions suitable for MCDA primer design were identified from the
171 genome alignment of 1,216 SARS-CoV-2 strains. Two regions, designated as region 1 and 2,
172 belonged to the ORF1ab gene at NC_045512.2 position 515-831 and 12968-13288
173 respectively. One region, designated as region 3, corresponded to the N-gene at
174 NC_045512.2 position 28345-28647. Four MCDA primer sets for each region was designed
175 and evaluated (Supplementary Table 1).

176

177 Each MCDA primer set was initially tested at 3 isothermal amplification temperatures (60°C,
178 63°C and 65°C) using 1000 DNA copies/reaction as the starting template. As seen in Figure
179 1, regardless of the primer sets used, the slowest amplification time was observed at 60°C.
180 Amplification at 63°C and 65°C were similar and 65°C was chosen as the isothermal
181 amplification temperature used.

182

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183 To maintain MCDA assay robustness against SNPs which may affect MCDA primer binding
184 and amplification efficiency, primer sets from two different regions were chosen for further
185 development as a duplex assay. Amplification of region 2 was the slowest for all primer sets
186 (Figure 1 D-F) compared with region 1 and 3, taking between 15-25 minutes at 65°C. Primer
187 sets in region 2 also had very high variation between technical replicates (data not shown).
188 Therefore region 2 was removed from further evaluation.
189

190 Region 3 amplification of the N gene was the fastest with primer set 2 followed closely by
191 primer set 3 (Figure 1G-I). Primer set 1 and 4 were the slowest for region 3 and were
192 therefore eliminated from further testing. We also observed that primer set 2 had tighter
193 technical replicates compared to primer set 3 (data not shown), thus region 3 primer set 2 was
194 chosen as our final MCDA primer set for further sensitivity and specificity testing.
195

196 Within region 1, primer set 3 was the slowest with fluorescence appearing at ~35 min (Figure
197 1C). This primer set was removed from further consideration. Primer set 1 was the fastest
198 primers to amplify region 1 and was chosen for inclusion in our MCDA assay.
199

200 Therefore, the final primer sets chosen for MCDA SARS-CoV-2 detection was region 1
201 (ORF1ab) primer set 1 and region 3 (N gene) primer set 2 (Figure 2). Both primer sets
202 showed no non-specific amplification when tested against human and microbial community
203 genomic DNA.
204

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205 **Sensitivity and time to detection comparison of MCDA, LAMP and rt-PCR**

206 The sensitivity and speed for MCDA, LAMP and rt-PCR were then compared for two SARS-
207 CoV-2 genes. For the N gene (region 3), detection by MCDA was consistently faster than
208 LAMP, by ~10-13 minutes, for most DNA dilutions tested (Table 1). The average fastest
209 detection time for MCDA was 5.2 minutes at 10,000 copies/µl while for LAMP it was 15
210 minutes. MCDA also had higher sensitivity with the limit of detection at 100 copies/µl while
211 for LAMP it was 500 copies/µl. This limit of detection was equivalent to a rt-PCR Ct value
212 of 32.4 and 30.3. respectively. A greater number of sporadic detections for higher dilutions
213 were also observed for MCDA compared to LAMP. MCDA was also significantly faster than
214 rt-PCR by ~20 minutes for lower dilutions and 10 minutes for higher dilutions. At 10,000
215 copies/µl , the detection time for rt-PCR was 23 minutes. However, rt-PCR had the highest
216 sensitivity with the limit of detection at 10 copies/µl and sporadic amplification at 1 copy/µl.
217 Similar results were also observed using RNA template with rt-PCR being the most sensitive
218 technique, detecting RNA at 10^{-6} dilution. For MCDA, the lowest RNA dilution detected was
219 10^{-4}, with only sporadic detection for LAMP at this dilution.

220

221 For ORF1ab, LAMP was more sensitive than MCDA with the limit of detection at 50
222 copies/µl and had more sporadic detection at higher dilutions (Table 2). The limit of
223 detection for MCDA was 500 copies/µl . LAMP was also slightly faster than MCDA by ~1-3
224 minutes for concentrations above the limit of detection. The fastest time to detection for
225 ORF1ab LAMP was 8.4 minutes while for MCDA it was 9.7 minutes.

226

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227 **Discussion**

228 Our results showed that MCDA is the fastest nucleic acid amplification method tested for
229 SARS-CoV-2 detection with detection of the N gene as fast as 5 minutes. However, this was
230 contingent on the gene targeted and the primer design with the NEB designed LAMP assay
231 for ORF1ab [3] showing similar speed to our equivalent MCDA ORF1ab assay.

232

233 rt-PCR remains the most sensitive nucleic acid amplification method for SARS-CoV-2
234 detection compared to MCDA and LAMP. This result is in agreement with previous LAMP
235 SARS-CoV-2 assays which showed rt-PCR having greater sensitivity[9,10]. The limit of
236 detection for our MCDA N gene assay was 100 copies/µl or an equivalent N gene average Ct
237 value of 32.4 (Table 1). The median rt-PCR Ct value in 324 clinical COVID-19 samples from
238 a range disease severity was found to be 31.15 in Singanayagam et al.[11] while in Passomsub
239 et al.[12] the median N gene Ct value in saliva samples and nasopharyngeal/throat samples were
240 31.8 and 30.5, respectively. This suggests that our MCDA assay has the potential to detect
241 SARS-CoV-2 but with lower sensitivity and consistent with our comparison using synthetic
242 templates. Furthermore, Lamb et al.[7] developed a COVID-19 LAMP assay with a limit of
243 detection of 0.08 fg or an equivalent rt-PCR Ct value of 30.3 and were able to validate their
244 LAMP assay in 19/20 positive clinical COVID samples. Our MCDA assay has increased
245 sensitivity and speed compared to LAMP, suggesting that MCDA has the potential for similar
246 applications as LAMP with better sensitivity and speed.

247

248 For MCDA, this is the first study to directly benchmark the speed and sensitivity of MCDA
249 to rt-PCR against the same targets. Previous MCDA studies only compared gel-based PCR[4],
250 different rt-PCR gene targets[13,14] or used rt-PCR sensitivity results previously reported in

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251 other studies[4,5] (as 100 copies in different studies may not be equivalent due to pipetting
252 differences, differences in the method used to measure nucleic acid concentration (nanodrop
253 vs qubit) or differences in machine calibration, etc.). In order to benchmark different nucleic
254 acid techniques, we used and recommend the same reaction volume, same machine, same
255 DNA standards and aliquots, and where possible the same run is used.

256

257 This study found that different nucleic acid amplification methods offer different advantages
258 and this should be considered depending on the application. rt-PCR was the most sensitive
259 method tested and should remain the gold standard for SARS-CoV-2 detection. However, the
260 portable nature and speed of MCDA makes it suitable for settings where rt-PCR would be too
261 slow. Although the fastest time to detection for MCDA is ~5 minutes, MCDA amplification
262 should be performed for at least 20 minutes to ensure reliable results for negative samples
263 while for rt-PCR, the current amplification time, not including reverse transcription, is 30-40
264 minutes. Additionally, reverse transcription and amplification for MCDA and LAMP can
265 occur simultaneously. This removes the need to sequentially perform an initial 20 min reverse
266 transcription step prior to amplification as required for rt-PCR, making MCDA even faster.
267 Therefore, it is estimated that the total time saved using MCDA compared to rt-PCR is 30-40
268 minutes. An additional advantage of MCDA is that it uses the same Bst polymerase and
269 reverse transcriptase as LAMP, which are more resistant against inhibitors than rt-PCR[15].
270 LAMP has been shown to amplify SARS-CoV-2 RNA extracted using simple extraction
271 procedures such as boiling[16-18]. Therefore, it is anticipated that MCDA can also be used to
272 detect SARS-CoV-2 RNA extracted using these same procedures.

273

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274 MCDA (and other isothermal amplification methods) is less sensitive than rt-PCR, making it
275 less attractive to develop it further as a clinical diagnostic test. However, there may be
276 situations where these methods will be useful such as rapid screening of samples with high
277 viral RNA content. The addition of a colorimetric dye instead of a fluorescent dye can
278 further simplify MCDA for rapid screening. Further studies in a variety of settings will be
279 required to determine where MCDA and other isothermal nucleic acid methods can offer an
280 advantage in certain settings where rapid test turnaround time or test simplicity is paramount.

281 Competing interests statement

282 The authors declare no competing interests.

283 Author Contributions

284 RL conceived the study. LDWL performed the experiments, analysed the results and drafted
285 the manuscript. MP performed the MCDA target selection and BLAST. XZ and LL designed
286 the MCDA primers. All authors provided critical revision of the manuscript.

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289 Data availability

290 All data generated or analysed during this study are included in this published article (and its
291 Supplementary Information files).

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349

350 # Figures

351 **Figure 1:** Initial evaluation of MCDA primer sets at 3 different isothermal amplification
352 temperature (60°C, 63°C and 65°C). Four MCDA primer sets were designed for each target
353 region chosen. **A-C**: Region 1 ORF1ab: 515-831 **D-E:** Region 2 ORF1ab: 12968-13288 **F-H**:
354 Region 3 N gene:28345-28647.

355

356 **Figure 2:** The nucleotide sequences and position of the final **(A)** ORF1ab and **(B)** N gene
357 COVID-19 MCDA primer sets chosen in this study. Right and left arrows show sense and
358 complementary sequences, respectively while coloured text indicate the position of primers:

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359 F1/F2 displacement primers in purple, P1/P2 primers in green, C1/C2 amplification primers

360 in blue, D1/D2 amplification primers in yellow and R1/R2 amplification primers in red.

361 # Tables

362 Table 1: Comparison of the sensitivity and time to detection for MCDA, LAMP and rt-PCR targeting the N gene from 3 independent runs.

DNA copies per µl	MCDA average detection time (min)	MCDA reps[$]	LAMP average detection time (min)	LAMP reps[$]	rt-PCR average Ct value	rt-PCR average detection time (min)	rt-PCR rep[$]
1	NA	NA	NA	NA	43.93±1.34	38.61±1.11	3/9
10	19.3 ±17.6	5/9	20.9 ±0.4	2/9	**35.5 ±0.6***	**31.6 ±0.47***	**9/9***
25	20.4 ±22.4	4/9	26.7 ±11.4	3/9	34.2 ±0.03	30.5 ±0.03	9/9
50	7.8 ±1	6/9	20.3 ±1	6/9	33.0 ±0.25	29.5 ±0.21	9/9
100	**10.2 ±3.2***	**9/9***	23.5 ±8.6	5/9	32.4 ±0.22	29.0 ±0.19	9/9
5,00	6.5 ±0.1	9/9	**17.5 ±0.6***	**9/9***	30.3 ±0.68	27.3 ±0.57	9/9
1,000	6.2 ±0.2	9/9	17.3 ±0.7	9/9	29.2 ±0.26	26.3 ±0.22	9/9
10,000	5.2 ±0.1	9/9	15.0 ±0.4	9/9	25.7 ±0.06	23.4 ±0.05	9/9

363 NA = no amplification detected

364 *Limit of detection

365 [$] reps: 3 runs with 3 technical replicates = 9 replicates.

366

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367 Table 2: Comparison of the sensitivity and time to detection for MCDA and LAMP targeting ORF1ab from 3 independent runs.

DNA copies per reaction	MCDA average detection time (min)	MCDA reps$	LAMP average detection time (min)	LAMP reps$
10	55.3	1/9	17.1 ±6.3	3/9
25	46.4 ±15	3/9	11.8 ±0.5	5/9
50	20.2 ±9	8/9	**12.2 ±2.1***	**9/9***
100	13.6 ±3.9	6/9	10.4 ±0.8	8/9
500	**12.6 ±1.3***	**9/9***	9.7 ±0.2	9/9
1,000	11.2 ±0.3	9/9	9.5 ±0.1	9/9
10,000	9.7 ±0.3	9/9	8.4 ±0.1	9/9

368 *Limit of detection

369 $ reps: 3 runs with 3 technical replicates = 9 replicates.

370

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371 ## Supplementary Materials

372 **Supplementary Table 1:** List of MCDA, LAMP and rt-PCR primers used in this study. Bolded MCDA primer names are primers shared
373 between 2 or more primer sets. * indicate primers used in rt-PCR for confirmation of complete DNA removal from transcribed RNA. Red text
374 indicates the final primer sets chosen for the MCDA SARS-CoV-2 assay.

375 **Supplementary Table 2:** List of synthesised gene fragments used as DNA/RNA template for MCDA, LAMP and rt-PCR. Blue indicates
376 universal M13 adapters while red depicts the sequence for T7 promoter.

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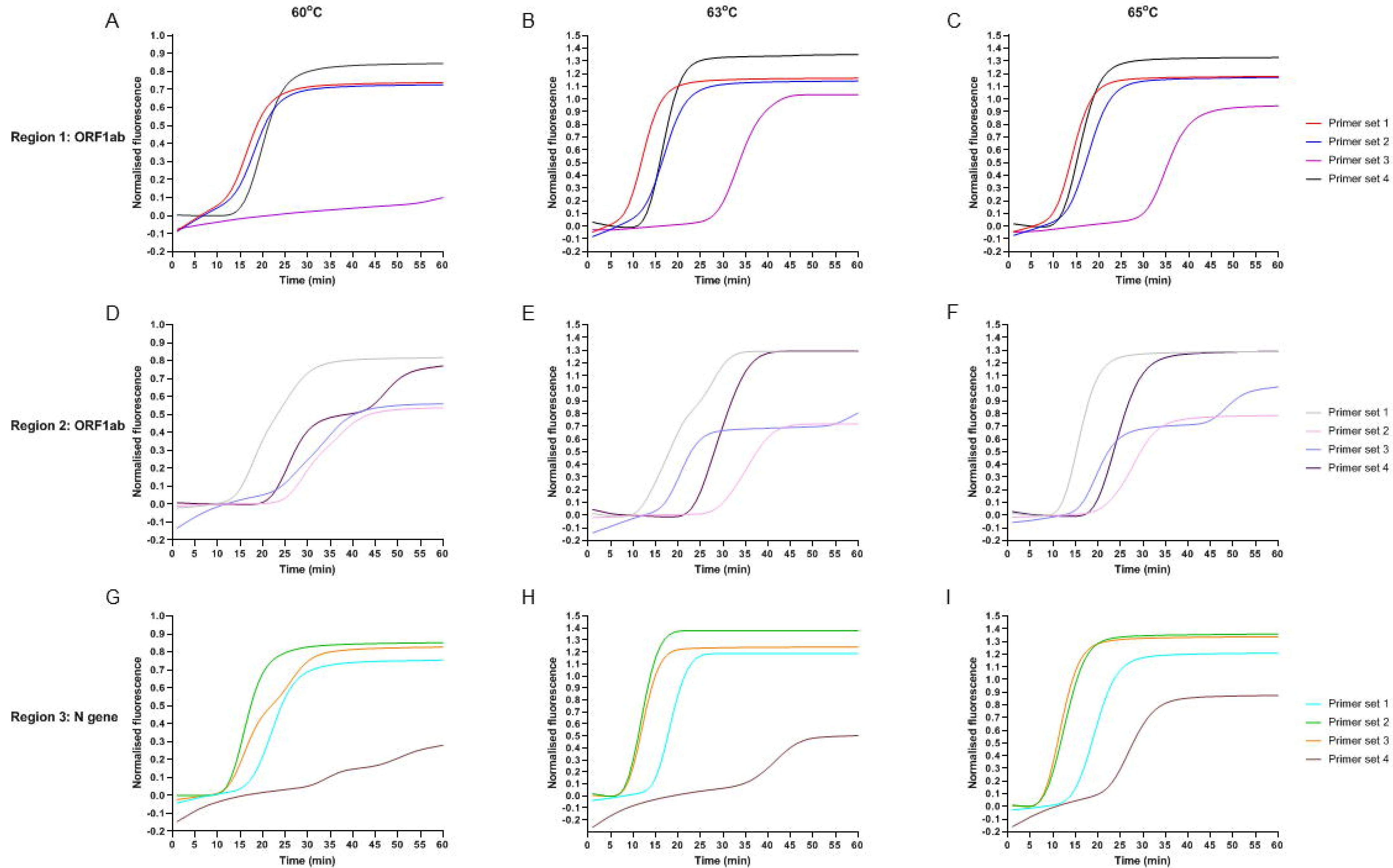

60°C
63°C
65°C
A
B
C
D
E
F
G
H
I
Region 1: ORF1ab
Region 2: ORF1ab
Region 3: N gene
Normalised fluorescence
Time (min)
Primer set 1
Primer set 2
Primer set 3
Primer set 4

A NC_045512.2: 515-831 (ORF1ab)

```
  1 TTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACAC

 61 TTGGTGTCCTTGTCCCTCATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTC
                   >>>>>>>>F1>>>>>>>>>           >>>>>>>>P1>>>>>

121 GTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTG
    >>>>>> <<<<<<<D1<<<<<<<<<<< <<<<<<<<<<<<<<C1<<<<<<<<<<<<<<<<

181 ACTTAGGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTA
    <<<R1<<<<<<<<  >>>>>>>>>>R2>>>>>>>>>      >>>>>>>>>>C2>>>>>>

241 AACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCATACACTC
    >>>>>>>>>>>>>>>>D2>>>>>>>>>><<<<<<<<P2<<<<<<<<<<   <<<<<<<<<

301 GCTATGTCGATAACAA
    <<<<F2<<<<<
```

B NC_045512.2: 28345-28647 (N gene)

```
  1 GGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGT
                                           >>>>>>>>F1>>>>>>>>>

 61 TTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTT
             >>>>>>>>P1>>>>>>>>>>><<<<<<<<<<<D1<<<<<<<    <<<<<<

121 AAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATT
    <C1<<<<<<<<<<<<<<<  <<<<<<<<R1<<<<<<<<<<<     >>>>>>>R2>>>>>

181 GGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAAAATGAAAGATCTC
    >>>>>> >>>>>>>>>>>>>C2>>>>>>>>>>>>>>>>>>D2>>>>>>>>>>      <<<

241 AGTCCAAGATGGTATTTCTACTACCTAGGAACTGGCCAGAAGCTGGACTTCCCTATGGT
    <<<<<<P2<<<<<<<<<<< <<<<<<F2<<<<<<<<<<

301 GC
```