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Implementation of an in-house real-time reverse transcription-PCR assay for the rapid detection of the SARS-CoV-2 Marseille-4 variant

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8

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ABSTRACT

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Introduction. The SARS-CoV-2 pandemic has been associated with the occurrence since summer 2020 of several viral variants that overlapped or succeeded each other in time. Those of current concern harbor mutations within the spike receptor binding domain (RBD) that may be associated with viral escape to immune responses. In our geographical area a viral variant we named Marseille-4 harbors a S477N substitution in this RBD.

Materials and methods. We aimed to implement an in-house one-step real-time reverse transcription-PCR (qPCR) assay with a hydrolysis probe that specifically detects the SARS-CoV-2 Marseille-4 variant.

Results. All 6 cDNA samples from Marseille-4 variant strains identified in our institute by genome next-generation sequencing (NGS) tested positive using our Marseille-4 specific qPCR, whereas all 32 cDNA samples from other variants tested negative. In addition, 39/42 (93%) respiratory samples identified by NGS as containing a Marseille-4 variant strain and 0/26 samples identified as containing non-Marseille-4 variant strains were positive. Finally, 2,018/3,960 patients SARS-CoV-2-diagnosed in our institute, 10/277 (3.6%) respiratory samples collected in Algeria, and none of 207 respiratory samples collected in Senegal, Morocco, or Lebanon tested positive using our Marseille-4 specific qPCR.

Discussion. Our in-house qPCR system was found reliable to detect specifically the Marseille-4 variant and allowed estimating it is involved in more than half of our SARS-CoV-2 diagnoses since December 2020. Such approach allows the real-time surveillance of SARS-CoV-2 variants, which is warranted to monitor and assess their epidemiological and clinical characteristics based on comprehensive sets of data.

TEXT

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1. Introduction

Since the onset of the SARS-CoV-2 pandemic in December 2019 in China, almost 100 million cases have been reported worldwide as on January 28th, 2021 (<https://www.ecdc.europa.eu/en/covid-19-pandemic>). This has been associated with the occurrence since summer 2020 of several viral variants that overlapped or succeeded each other in time [1-3]. Those of current concern harbor mutations within the spike glycoprotein, particularly within the spike receptor binding domain (RBD) that leads to viral entry into host cells by binding to the ACE2 receptor (Figure 1) [4]. Such SARS-CoV-2 variants include the 20I/501Y.V1 [3], 20H/501Y.V2 [5], and 20J/501Y.V3 [6] strains that harbor a N501Y substitution in the spike RBD and were reported in the UK and in South Africa, as highly transmissible, and in Brazil, respectively. In our geographical area we detected 10 viral variants since June 2020 [1]. One of them, we named Marseille-4, harbors a S477N substitution in the spike RBD that has been associated with an improved binding affinity to ACE2 [6] and a broad resistance to monoclonal neutralizing antibodies [7]. It predominates in Marseille since August 2020, has been reported to spread in Europe since early summer and was classified as the Nextstrain 20A.EU2 lineage [1, 2]. The continuous emergence of new SARS-CoV-2 variants, including some of substantial concern regarding their transmissibility and their possible ability to evade immune responses [8-10], warrants to set up strategies for their detection and surveillance. SARS-CoV-2 incidence is currently substantial in several countries including France, and in our institute we for instance diagnose >100 new cases daily. Therefore, alternative strategies to sequencing are useful for variant screening. We aimed to implement an in-house one-step real-time reverse transcription-PCR (qPCR) assay that specifically detects the SARS-CoV-2 Marseille-4 variant.

50 **2. Material and methods**

51 SARS-CoV-2 genomes from our institute sequence database and from the GISAID
52 database (<https://www.gisaid.org/> [11]) were used to design a primer pair and a hydrolysis
53 probe. These sequences target a fragment of the nsp4 gene that contains nucleotide position
54 9,526 of the viral genome [in reference to genome GenBank Accession no. NC_045512.2
55 (Wuhan-Hu-1 isolate)] where is located a hallmark mutation G>U of the SARS-CoV-2
56 Marseille-4 variant. The sequences of the qPCR primers and probe are shown in Table 1. The
57 qPCR was performed by adding 5 µL of extracted viral RNA to 15 µL of reaction mixture
58 containing 5 µL of 4X TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific,
59 Grand Island, NY, USA), 0.5 µL of forward primer (10 pmol/µL), 0.5 µL of reverse primer
60 (10 pmol/µL), 0.4 µL of probe (10 pmol/µL), and 8.6 µL of water. PCR conditions were as
61 follows: reverse transcription at 50°C for 10 min, then a hold at 95°C for 20 sec followed by
62 40 cycles comprising a denaturation step at 95°C for 15 sec and a hybridization-elongation
63 step at 60°C for 60 sec. This qPCR was run on a LC480 thermocycler (Roche Diagnostics,
64 Mannheim, Germany).

65

66 **3. Results**

67 Firstly, we tested a panel of 38 cDNA samples from each of the 10 variants named
68 Marseille-1 to Marseille-10 that we identified by genome next-generation sequencing (NGS)
69 and circulated since summer 2020 in our geographical area (6 from Marseille-4 strains, 5 from
70 Marseille-5 strains, 5 from Marseille-3 strains, 4 from Marseille-1 strains, and 3 from strains
71 classified in each of the variants Marseille-2, Marseille-6, Marseille-7, Marseille-8, Marseille-
72 9, and Marseille-10) [1]. All 6 Marseille-4 samples tested positive whereas all 32 samples
73 from other variants tested negative. Secondly, we tested 42 samples identified in our institute
74 by genome NGS [1] as being from patients infected with a SARS-CoV-2 Marseille-4 variant:

75 39 of them (93%) were positive using our Marseille-4 specific qPCR. Thirdly, we tested 26
76 samples identified by next-generation genome sequencing as containing SARS-CoV-2 strains
77 that were not Marseille-4 variants (including 17 N501Y variants, 5 Marseille-2 variants, 3
78 clade 20A strains and 1 clade 20C strain): none of them were positive using our Marseille-4
79 specific qPCR. Positive and negative predictive values of Marseille-4 detection by our qPCR
80 were 100% and 90%, respectively. Finally, we tested with our Marseille-4 specific qPCR the
81 respiratory samples from 4,339 patients SARS-CoV-2-diagnosed in our institute. None of 22
82 patients' samples collected in June, 20 (5.6%) of 357 patients' samples collected in July, and
83 2,018 (51%) of 3,960 patients' samples collected in December 2020 and January 2021 tested
84 positive (Figure 2). These results are congruent with those obtained based on genome NGS
85 that showed that the Marseille-4 variant emerged in our geographical area in July and has
86 been predominant since August 2020 [1]. In addition, we found that 10 (3.6%) of 277
87 respiratory samples collected in Algeria in September-October tested positive using our
88 Marseille-4 specific qPCR, while none of 94 respiratory samples collected in Senegal in
89 September-October, of 94 samples collected in Morocco in November 2020, and of 19
90 samples collected in Lebanon in October 2020, tested positive.

91

92 **4. Discussion**

93 Our in-house qPCR system was found reliable to detect specifically the Marseille-4
94 variant and allowed estimating it is involved in more than half of our SARS-CoV-2 diagnoses
95 since December 2020. This assay is currently routinely used in our clinical microbiology and
96 virology laboratory to screen systematically all samples found SARS-CoV-2-positive using
97 the first-line qPCR diagnosis assay, which allows the real-time classification of viral strains in
98 about half of the diagnoses (Figure 2). In case of negativity of this Marseille-4 specific qPCR,
99 samples are tested using alternative qPCR assays that are specific to other variants that

100 circulate at a lower incidence level than the Marseille-4 variant, or they are submitted to next-
101 generation sequencing in case of cycle threshold value (Ct) <18 with the SARS-CoV-2 qPCR
102 diagnosis test [1, 12]. Such approach based on qPCR assays targeting specifically SARS-
103 CoV-2 variants allows their real-time surveillance, which is warranted to monitor and assess
104 their epidemiological and clinical characteristics based on comprehensive sets of data. In
105 addition, in-house qPCR assays can be implemented rapidly, easily and at low cost on various
106 open qPCR microplate platforms, which may allow adapting continuously the diagnosis
107 strategies to the emergence and dynamics of SARS-CoV-2 variants.

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110 **Credit authorship contribution statement**

111 Conceived and designed the experiments: DR, PC. Contributed materials/analysis tools: MB,
112 PEF, LH, JD, LP, IB, AC, HTD, PB, CS, RS, EA, SM, PC. Analyzed the data: MB, PC, DR.
113 Wrote the paper: PC, MB, DR.

114

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122

123 **Declaration of Competing Interest**

124 The authors have no conflicts of interest to declare. Funding sources had no role in the design

125 and conduct of the study; collection, management, analysis, and interpretation of the data; and
126 preparation, review, or approval of the manuscript.

127

128 **Ethics**

129 This study has been approved by the ethics committee of our institution (N°2020-029).

130

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132 This manuscript text has been edited by a native English speaker.

133

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FIGURES

Figure 1. Three-dimensional structure of the SARS-CoV-2 spike protein showing amino acid substitutions and deletions for major SARS-CoV-2 variants including the Marseille-4 variant. Structure prediction was performed using the Phyre2 web portal (<http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>) and visualized using the Pymol tool v.1.8 (<https://pymol.org/2/>). Amino acid substitutions and deletions are showed with a country flag for the variants first detected in UK (20I/501Y.V1), South Africa (20H/501Y.V2) and Brazil (20J/501Y.V3), or with the label “Marseille-4.”

Figure 2. Number and proportion of Marseille-4 variants detected by qPCR in respiratory samples from patients diagnosed with SARS-CoV-2 in our institute. The graph shows the weekly numbers of patients tested by our Marseille-4 specific qPCR assay (grey bars) and the weekly numbers (red bars) and proportions (red curve) of positive tests in January 2021. Systematic testing of RNA extracts obtained from nasopharyngeal samples of patients newly-diagnosed with SARS-CoV-2 was implemented in our institute from January 1st, 2021 using our Marseille-4 specific qPCR assay.

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TABLE

193

194 **Table 1. Primers and probe of the Marseille-4 variant-specific qPCR**

195

Name	Sequence (5'-3')	Positions *
<i>Primers:</i>		
Pri_IHU_C4_5_MBF	GAGGTTTAGAAGAGCTTTTGGTGA	9,460-9,483
Pri_IHU_C4_5_MBR	CCAGGTAAGAATGAGTAAACTGGTG	9,549-9,573
<i>Probe (6FAM-labelled):</i>		
Pro_IHU_C4_5_MBP	CCTTAT <u>TT</u> CATTCACTGTACTCTG	9,520-9,543

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197 * in reference to SARS-CoV-2 genome GenBank Accession no. NC_045512.2 (Wuhan-Hu-1

198 isolate). The nucleotide carrying the mutation specific to the Marseille-4 variant is covered

199 by the probe and underlined.

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Fig. 1

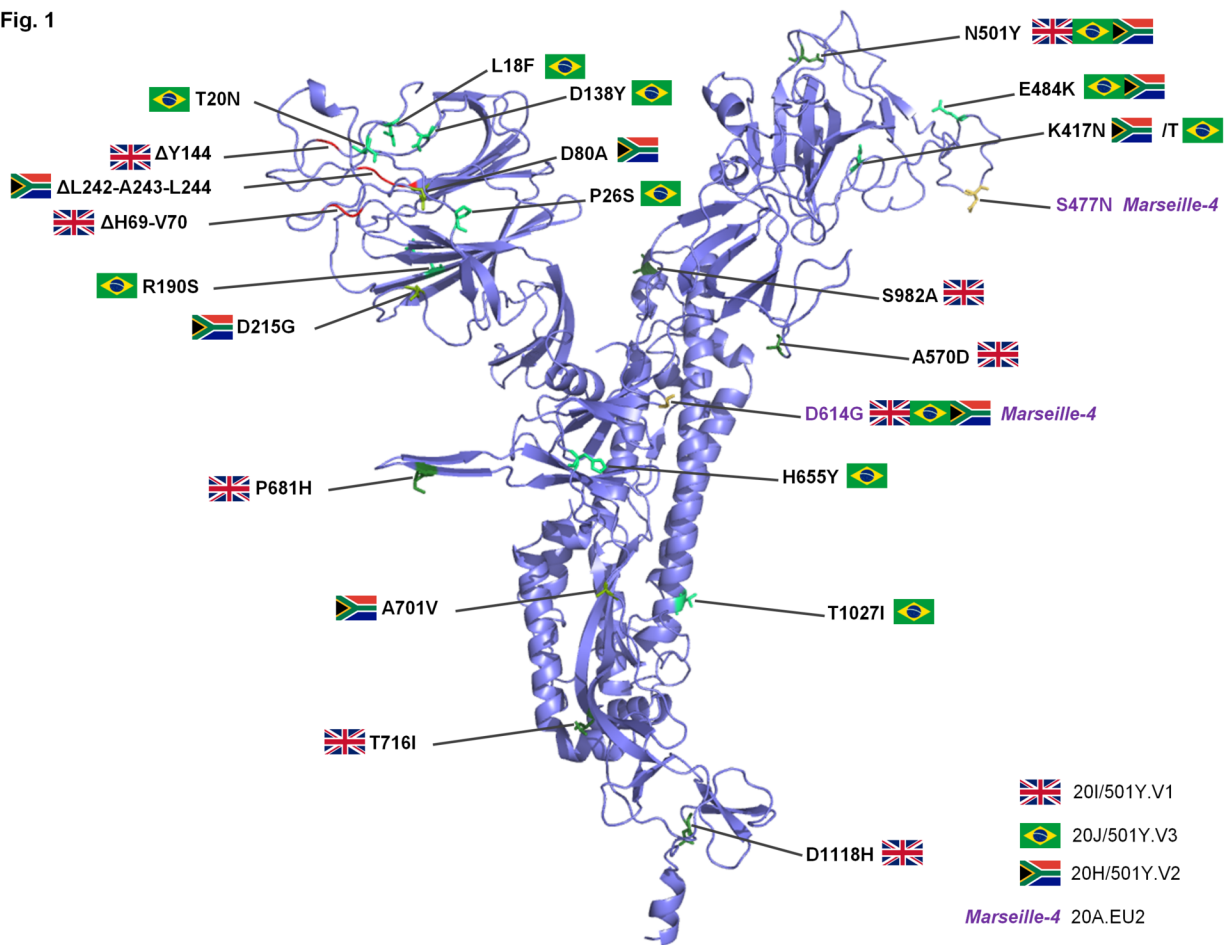


Fig. 2

