

IHU Méditerranée Infection

In-house one-step real-time reverse transcription-PCR (qPCR) assay that specifically detects the SARS-CoV-2 Marseille-4 variant (a.k.a. Nextstrain clade 20A.EU2)

This qPCR system targets nucleotide positions 9,460-9,543 in reference to genome NC_045512.2 (Wuhan-Hu-1 isolate) within the nsp4 gene.

Table. Primers and probe

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	<u>CCTTATTT</u> CATTCACTGTACTCTG	9,520-9,543

* in reference to genome NC_045512.2 (Wuhan-Hu-1 isolate). The nucleotide specific of the Marseille-4 variant is covered by the probe and underlined.

qPCR conditions

The qPCR can be performed by adding 5 μ L of extracted viral RNA to 15 μ L of reaction mixture containing 5 μ L of 4X TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Grand Island, NY, USA), 0.5 μ L of forward primer (10 pmol/ μ L), 0.5 μ L of reverse primer (10 pmol/ μ L), 0.4 μ L of probe (10 pmol/ μ L), and 8.6 μ L of water. PCR conditions are as follows: reverse transcription at 50°C for 10 min, then a hold at 95°C for 20 sec followed by 40 cycles comprising a step at 95°C for 15 s and a step at 60°C for 60 s. This qPCR was run on a LC480 thermocycler (Roche Diagnostics, Mannheim, Germany).