LamPORE SARS-CoV-2 diagnosis and genotyping: a preliminary report.

Morsli, M.¹,², Anani, H.², Bréchard, L.², Delerce, J.²,
Bedotto, M.³, Fournier, P.-E.²,³, Drancourt, M.¹,²

1. Aix-Marseille-Univ., IRD, MEPHI, IHU Méditerranée Infection, Marseille, France.
2. IHU Méditerranée Infection, Marseille, France.
3. Aix Marseille Univ., IRD, AP-HM, SSA, VITROME, Marseille, France.

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Corresponding author: Michel DRANCOURT (michel.drancourt@univ-amu.fr)
Dear Editor.

Direct diagnosis of COVID-19 is mainly based on the reverse-transcription PCR (RT-PCR) detection of the SARS-COV-2 RNA (1) (2). The LamPORE process has been recently developed for detecting the viral genome by the reverse-transcription loop-mediated amplification (RT-LAMP) and sequencing the RT-LAMP product on LamPORE instrument (Oxford Nanopore, Oxford, UK) (3). Here, 264 nasopharyngeal swabs routinely submitted to BGI’s Real-Time Fluorescent RT-PCR kit (BGI, Wuhan, China), (61 positive and 203 negative samples) at the IHU Méditerranée Infection, Marseille, France, were investigated in parallel by the LamPORE procedure. Briefly, the LamPORE procedure combines an isothermal amplification step for 30 minutes at 65°C, performed in a 50-µL volume containing 20 µL RNA on Kingfisher instrument, using MagMax™ Viral/Pathogen Kit (ThermoFisher, St. Austin, USA), 5 µL priming Mix and 25 µL of LamPORE master Mix, targeting three SARS-COV-2 genes: ORF1a, the envelope (E) and nucleocapsid (N) genes in addition to the human actin mRNA as internal control. Further, 2 µL of each amplified sample were engaged in LamPORE library preparation as previously described (3) and the library was sequenced on LamPORE instrument for one hour. LamPORE assays incorporated water as negative control, provided by the manufacturer. LamPORE diagnosis results interpretation positive and negative tests automatically generated in a pdf file as follows: positive test when the sum of reads generated by the three targets ≥ 50; negative test when number of reads < 20; inconclusive test when number of reads between 20 and 50. Strictly using the interpretation algorithm proposed by the manufacturer, all the 61 nasopharyngeal swabs routinely positive for SARS-COV-2 were found positive by LamPORE (100%
sensitivity) but 23/203 of RT-PCR negative nasopharyngeal swabs detected positive by LamPORE (88.7% specificity). Analyzing these 23 discordant results, we observed that for a result to be consistent with RT-PCR, the sum of the 3 targets reads > 50 and the multiplication of the N (reads) of the 3 targets > 0 was required. Applying this new interpretation rule allowed to achieve 100% sensitivity and 100% specificity, in the 61-sample collection we investigated. This new interpretation rule consisted in eliminating false positives by applying the “=SI(ET((C2+D2+E2>50);(C2*D2*E2>0));"pos","neg")” formula directly in the Excel file. In a second step, we used sequences to detect SARS-CoV-2 genotype 4, the most prevalent genotype in the Marseille area in the considered period. Indeed, LamPORE theoretically generates substitutions G28,975T and G29,399A in the nucleocapsid gene, among the 13 mutations specific for genotype 4 (Figure 1). Here, LamPORE detected two genotype 4, based on two sequences exhibiting only the G28,975T mutation and one sequence exhibiting the mutation G29,399A.

Although preliminary, data here reported confirm that LamPORE is an appropriate method for the rapid direct detection of SARS-CoV-2 RNA in nasopharyngeal swabs, with a capacity of 480 tests per hour, depending on adoption of the interpretation rule here reported; and offer promise for one-shot genotyping of SARS-CoV-2 depending on further experimental improvements, offering a new alternative way for SARS-COV-2 detection and genomic surveillance.
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Ethics

Only nasopharyngeal residual fluid left from standard-of-care clinical laboratory testing was used. All specimens had been referred to our laboratory for diagnostic purposes between September 1st and November 1st, 2020. The study was validated by our Institute’s Ethical Committee under number 2020-016603.

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Conflicts of interest

Reagents for the LamPORE instrument have been provided by Oxford Nanopore Technology, Oxford, UK. Nevertheless, the supplier did not interfere in experimental plan, data interpretation, manuscript preparation and submission.

Author’s contribution.

MM, contributed to experimental design, realization of the work, data analysis, interpretation, and writing. AH, collected samples, data analysis, and writing. BL, organization of the work. DJ, Bioinformatics data analysis. BM, experimental design. PEF and DM, contributed to critically reviewing the manuscript, data interpretation,
coordinated and directed the work. All authors declare that they have read and approved the manuscript.
## References


Figure 1. LamPORE diagnosis strategy for SARS-COV-2 detection and genotyping. LAMP amplification and library preparation were followed by library sequencing on the LamPORE instrument. A genotyping step was added to specifically detect SARS-COV-2 genotype 4.