Received: 3 January 2021 | Accepted: 15 January 2021

DOI: 10.1002/jmv.26823

COMMENTARY

JOURNAL OF MEDICAL VIROLOGY WILEY

Will the emergent SARS-CoV2 B.1.1.7 lineage affect molecular diagnosis of COVID-19?

Juan D. Ramírez[1] | Marina Muñoz[1] | Luz H. Patiño[1] | Nathalia Ballesteros[1] | Alberto Paniz-Mondolfi[2,3]

[1]Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia

[2]Department of Pathology, Molecular and Cell-Based Medicine, Laboratory of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

[3]Instituto de Investigaciones Biomédicas IDB/Incubadora Venezolana de la Ciencia, Barquisimeto, Venezuela

Correspondence
Juan David Ramírez, Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia.
Email: juand.ramirez@urosario.edu.co

Abstract

As the coronavirus disease 2019 pandemic keep tackling global public health systems worldwide. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) genome keeps mutating. In that regard, the recent emergence of the B.1.1.7 lineage in the UK has called the attention of global authorities. One point of concern is that if this lineage can be detected by traditional molecular schemes for SARS-CoV-2 detection. Herein, we showed that this lineage does not affect the Berlin–Charité protocol but can challenge the available commercial kits directed to the Spike (S) gene. All efforts should be made to continue to monitor SARS-CoV-2 genomes for potential variants that can impair diagnostic testing and lead to false negative results.

KEYWORDS

coronavirus, DNA extraction, genetic variation, genetics, pathogenesis, phenotypic variation, research and analysis methods, virulence, virus classification

As the coronavirus disease 2019 (COVID-19) pandemic continues to spread globally, accuracy, and reliability of testing methods, as well as the quality and speediness of data reporting is essential to provide robust real time monitoring and surveillance data for disease control and prevention. Recent reports on the emergence of a new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral lineage (B.1.1.7) circulating in England have fueled speculations about its increased transmissibility and potential to affect molecular based testing methods. Even though this "variant under investigation" was first reported in late December in several areas of East, South East England, and London, evidence suggests that this variant could have been circulating since September 2020 and to presumably had arisen from a chronically infected patient.[1]

This new lineage is characterized by a repertoire of 17 mutations including 14 amino acid replacements and 3 in-frame deletions located in the ORF 1 a/b, ORF 8, Spike (S), and N gene regions.[1] Two of these mutations located in the spike (S-gene) have already proven to have potential biological implications. The N501Y mutation, which occurs within the receptor binding domain (RBD) region, has been shown to enhance binding affinity to human and murine ACE2 receptor, a key player in viral recognition and internalization. On the other hand, the 69-70 del has shown not only to play an important role in immunity but also has been associated to diagnostic failures on SARS-CoV-2 reverse-transcription polymerase chain reaction (RT-PCRs) assays targeting the S gene. In addition, a third mutation P681H occurring in close proximity to the furin cleavage site at the S1-S2 junction has been shown to have functional biological implications that may influence viral infectivity.[1] Ongoing studies suggest important epidemiological implications, including impact on virus transmissibility. In fact, current epidemiological modeling studies suggest a 56% increased transmissibility compared with other lineages, which may lead to a greater incidence and a larger number of projected hospitalizations and COVID-19 related deaths in 2021 when compared with 2020.[2] This situation has led again to the implementation of travel restriction in several countries including the UK, which has already implemented strict lockdown measures. However, despite such efforts, the B.1.1.7 lineage appears to have spread too far and has already been reported in 23 different countries thus precluding further containment and the global spread of this emerging variant.

One of the most pressing concerns from a diagnostic standpoint is how mutations of this new lineage may adversely affect performance

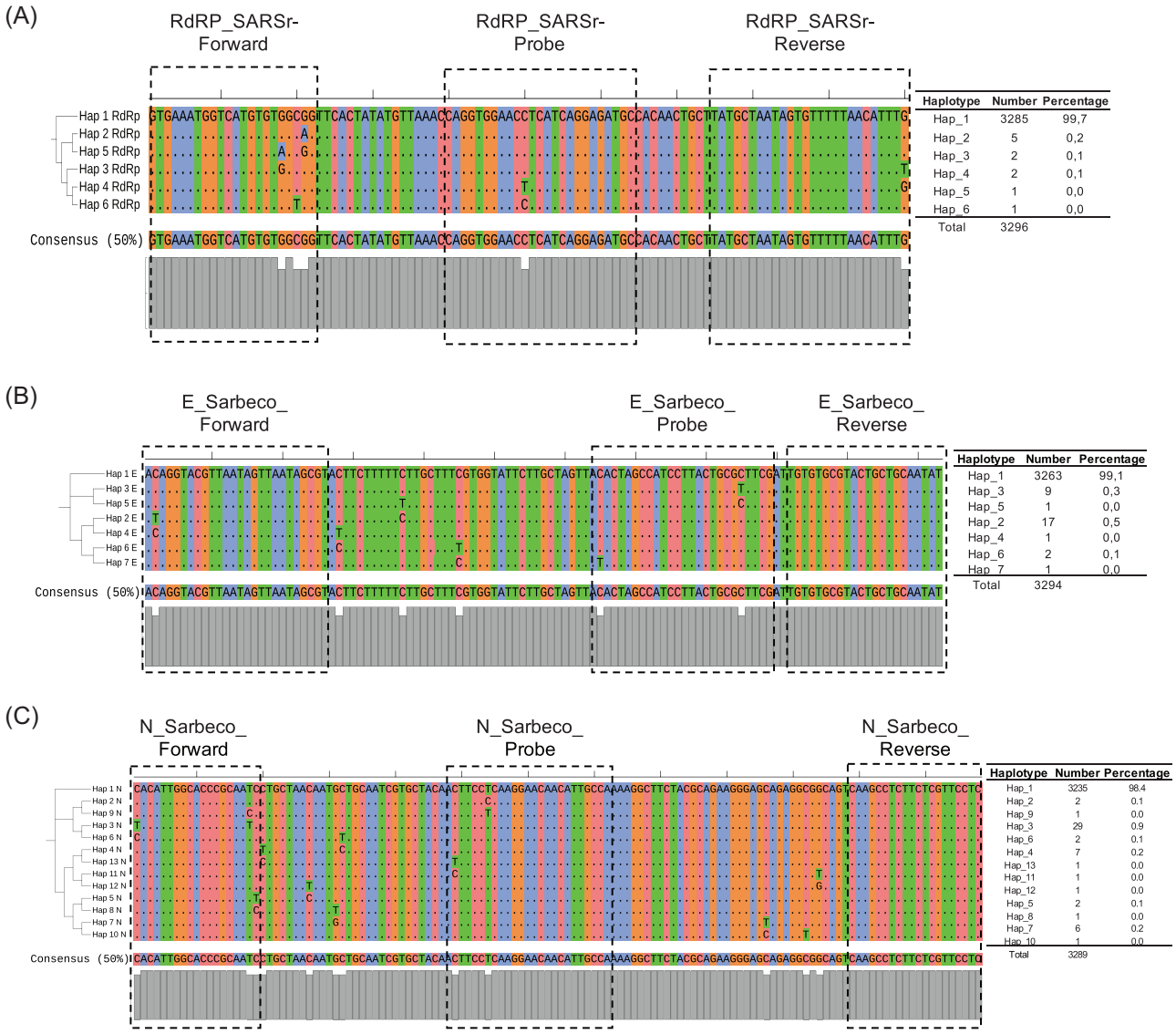

FIGURE 1 Multiple alignment of SARS-CoV2 B.1.1.7 lineage genomes and annealing sites of primers and probes for molecular diagnosis of COVID-19. (A) Annealing sites for RdRp gene. (B) Annealing sites for E gene. (C) Annealing sites for N gene. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

of currently available molecular tests for SARS-CoV-2 detection, particularly those targeting single positions of the viral genome. As a matter of fact, variants harboring the 69-70del have already been associated with "dropout" of the S gene targets across several diagnostic platforms.[1] Currently, several countries have implemented in-house testing based on the Berlin–Charité protocol[3] as well as other commercially available assays who do not disclose primers/probes sequences due to proprietary information policies. Also, analytical sensitivity and specificity between different RT-PCR assays may differ particularly in context of the emergence of new virus variants. Herein, we retrieved B.1.1.7 SARS-CoV-2 genomic sequences to assess if the described lineage specific mutations signature could affect primers/probes annealing within the Berlin–Charité protocol.

For this preliminary analysis we included all B.1.1.7. lineage public genome sequences available in GISAID for a total of 3296 full genomes. All three genes included in the Berlin protocol were evaluated: RdRp, E, and N genes with their corresponding primes/probes sequences as reported by Corman et al.[3] For all three genes, most of the assessed sequences fell into a predominant haplotype with a frequency greater than 98% in which the reference sequence NC045512-2-Wuhan-Hu-1 was also included. However, polymorphisms were identified in the amplified region that lead to the identification of additional haplotypes that although present at frequencies less than 1% did reveal presence of single nucleotide polymorphisms (SNPs) at oligo-binding site regions which could prevent accurate identification and lead to false negative results.

For the RdRp gene, six haplotypes were identified (Haplotype Diversity "Hd": 0.0067). SNPs were found in the haplotypes with lower frequencies, mainly in the first forward primer, with three variable positions at the 3′ end. In addition, a variable position was

found at the central region of the probe-annealing site and an additional in the reverse primer, corresponding to the first position at the 5′ end (Figure 1A). For the E gene, seven haplotypes were identified (Hd: 0.0187) depicting a variable position at the 5′ end of the forward primer plus two additional variable positions at the probe-annealing site (Figure 1B). For the N gene, 13 haplotypes were identified (Hd: 0.0325) with three variable sites at the forward primer-annealing site, two of them at the 3′ end, and two variable positions in the probe-annealing site (Figure 1C).

These results suggest that the B.1.1.7 lineage does not seem to impact dramatically diagnostic performance of assays based on the Berlin–Charité protocol considering that 98% of the sequences can be detected with current primers/probes sets. However, a few sequences do appear to have been missed by current primers and probes prompting reassessment and design of primers/probes sets as well as evaluation of reagents and cycling conditions.

Mutations and variations impairing accuracy and effectiveness of SARS-CoV-2 diagnostic assays have been recently documented.[4,5] A recent work from Vogels et al evaluating how the frequency of variants may impact efficiency of a number of qRT-PCR assays revealed the occurrence of a GGG > AAC mutation (genome positions: 28881-TO28883) overlapping on the 5′ of the Chinese CDC N forward primer.[6] Artesi et al have reported on the occurrence of a C-to-U transition at position 26340 of the SARS-CoV-2 genome linked to impaired detection of the E gene target on the Cobas (Roche) qRT-PCR platforms.[7] Furthermore, this mutation appears to have arisen independently at least four times, suggesting the possibility in its origin through different transmission chains.

Even though diagnostic detection of SARS-CoV-2 by RT-PCR using Berlin–Charité does not involve the spike (S) protein-encoding-gene as a target, the fact that 8 out of the 17 mutations defining the novel UK variant involve the S gene is a matter of concern that deserves further comments. In a recent press note dated December 22nd, DiaSorin Molecular has stated that an in silico analysis evaluating the multiple spike (S) and ORF1a/b mutations present in the emerging variant will not affect performance of the Simplexa™ COVID19 Direct assay, given that none of the reported mutations locate at the primers/probes targeted regions included in their assay.[8] Conversely, Thermo Fisher Scientific has already announced that the 69-70del mutation (S gene) will result in a "drop-out" of the S gene target, as this region is targeted through their *TaqPath* COVID-19 assay.[9] However, because *TaqPath* is a 3-gene target assay covering different regions on the S and N genes, it is unlikely that this will affect overall test performance. Moreover, S gene "drop out" may prove beneficial from an epidemiological standpoint as it may serve as a proxy indicator for detection of the emerging B.1.1.1.7 lineage. In fact, the European CDC has stated that for multitarget RT-PCR assays including the S gene, 'Spike drop out" maybe used as a surrogate marker for the 69-70del for variant detection in testing limited settings.[10]

In conclusion, it is expected that an increase in the frequency of variants could eventually impact testing of SARS-CoV-2 RT-PCRs assays for both in-house and commercially available assays. Such has been the case for the emerging UK variant which has accumulated an unprecedented repertoire of mutations in a very brief timeframe. This emphasizes the need to follow a multi-target approach interrogating different regions of the viral genome to build-in redundancy and increase test sensitivity. All efforts should be made to continue to monitor SARS-CoV-2 genomes for potential variants that can impair diagnostic testing and lead to false negative results.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmv.26823

ORCID

Juan D. Ramírez https://orcid.org/0000-0002-1344-9312

REFERENCES

1. Rambaut A, Loman N, Pybus O, Barclay W, Barrett J, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. Downloaded from https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563. Accessed December 24, 2020.
2. Davies N, Barnard R, Jarvis CI, Kucharski AJ, Munday J, et al. Estimated transmissibility and severity of novel SARS-CoV-2 variant of concern 202012/01 in England. *MedRxiv* https://doi.org/10.1101/2020.12.24.20248822
3. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR 2020. *Eur Surveill*. 2020;25:2000045.
4. Ramírez JD, Muñoz M, Hernández C, et al. Genetic diversity among SARS-CoV2 strains in South America may impact performance of molecular detection. *Pathogens*. 2020;9:580.
5. Wang H, Li X, Li T, et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *Eur J Clin Microbiol Infect Dis*. 2020;39:1629-1635.
6. Vogels CBF, Brito AF, Wyllie AL, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. *Nature Microbiol*. 2020;5:1299-1305.
7. Artesi M, Bontems S, Gobbels P, Franckh M, Maes P, et al. A recurrent mutation at position 26340 of SARS-CoV-2 is associated with failure of the E gene quantitative reverse transcription-PCR utilized in a commercial dual-target diagnostic assay. *J Clin Microbiol*. 2020:58. https://doi.org/10.1128/JCM.01598-20. Accessed December 24, 2020.
8. DIaSorin Molecular Note: Simplexa™ COVID-19 Direct Assay is Not Impacted by VUI – 12/01 SARS-CoV-2 Variant. file:///C:/Users/jdram/Downloads/2020.12.22%20-%20COVID-19%20Variant%20Letter%20-%20APPROVED.pdf. Accessed December 22, 2020.
9. Thermofisher report molecular diagnostic report COVID19. Downloaded from: (https://www.thermofisher.com/blog/behindthebench/thermo-fishers-covid-19-tests-designed-with-virus-mutations-in-mind/. Accessed December 24, 2020.
10. Center for Disease Prevention report 2020. Downloaded from https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-rapid-increase-sars-cov-2-variant-united-kingdom. Accessed December 24, 2020.

How to cite this article: Ramírez JD, Muñoz M, Patiño LH, Ballesteros N, Paniz-Mondolfi A. Will the emergent SARS-CoV2 B.1.1.7 lineage affect molecular diagnosis of COVID-19? *J Med Virol*. 2021;93:2566–2568. https://doi.org/10.1002/jmv.26823