Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

In response to: Multiple assays in a real-time RT-PCR severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak

As an RNA virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) lacks a robust proofreading mechanism during replication, increasing the likelihood of evolutionary mutants (van Dorp et al., 2020). This is exemplified by the recent emergence of new SARS-CoV-2 variants associated with increased viral load (Kidd et al., 2020), most notably, the VOC-202012/01 (UK) and 501Y.V2 (South Africa) variants. Genomic analysis of these variants shows the highest mutation frequency occurring predominantly in the spike gene (Kupferschmidt, 2020; Rambaut et al., 2020). Whether the expansion of these mutations is the result of selection or genetic drift is still under investigation (Tegally et al., 2020; Kupferschmidt, 2021). However, the rapid spread of these variants has evoked public concern regarding the reliability of diagnostic reverse transcription polymerase chain reaction (RT-PCR) assays.

We previously assessed the risk of loss of sensitivity of different published assays by the emergence of genetic variability through week 21 2020 (Penarrubia et al., 2020). We concluded that targeting more than one genomic region mitigates the risk of loss of sensitivity by the accumulation of mutations in the primer binding regions. We also demonstrated that the QIAstat-Dx Respiratory SARS-CoV-2 panel (Qiagen, Hilden, Germany) successfully identified >99% of SARS-CoV-2 genomes with 100% oligonucleotide coverage. The remaining <1% of genomes exhibited a single variation in the annealing region of one of the oligonucleotides. From these, 0.02% of sequences showed a mismatch in a critical position. In-vitro testing showed no effect on the limit of detection of the panel, highlighting the robustness of its multi-target approach to maintain nominal sensitivity (Penarrubia et al., 2020).

Table 1
QIAstat and NeuMoDx detection of novel SARS-CoV-2 variants.

SARS-CoV-2 variant	Mutation (amino acid change)	Reported public health impact	Geographical region	Impact on assay sensitivity	
				QIAstat-Dx Respiratory SARS-CoV-2 panel (RdRp and E assays)	NeuMoDx SARS-CoV-2 panel (Nsp2 and N assays)
VOC 202012/01	Deletion (spike): Δ69-70, Δ144 Substitution (spike): N501Y, A570D, P681H, T716I, S982A, D1118H	Report of increased transmissibility from the UK	Prevalent in parts of the UK, cases detected increasingly in other countries	No impact	No impact
501.V2	Substitution (spike): D80A, D215 G, E484 K, N501Y and A701V	Report of increased transmissibility from South Africa	Dominant in South Africa, two cases recently detected in the UK	No impact	No impact
Danish mink variant	Deletion (spike):Δ69-70 Substitution (spike): Y453F	Transmission from mink to humans and community spread confirmed. No changes in transmissibility reported	Prevalent in Denmark. Not detected elsewhere	No impact	No impact
Danish mink cluster 5	Deletion (spike):Δ69-70 Substitution (spike): Y453 F, I692 V, M1229I	Preliminary report of moderate reduction of neutralization by convalescent sera	Denmark, not observed since September 2020	No impact	No impact
Various variants with spike amino acid change N439K	Deletion (spike):often Δ69-70	Reports of minor reduction of neutralization by convalescent sera	Common in Czechia, Denmark and Ireland. Found in lower proportions in many countries	No impact	No impact
Next strain cluster 20A. EU1	Substitution (spike): A222V	Rapid increase in Spain and then the rest of the EU/EEA at the start of the second wave, probably due to random events and travel patterns	First observed in Spain. Most common variant in the EU/EEA	No impact	No impact

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Table 1 (*Continued*)

SARS-CoV-2 variant	Mutation (amino acid change)	Reported public health impact	Geographical region	Impact on assay sensitivity	
				QIAstat-Dx Respiratory SARS-CoV-2 panel (RdRp and E assays)	NeuMoDx SARS-CoV-2 panel (Nsp2 and N assays)
Next strain cluster 20A. EU2	Substitution (spike): S477N (nucleocapsid): A376T	Rapid increase in France at the start of the second wave, probably due to founder effects	First observed in France, and also prevalent in Belgium, Czechia, Denmark, Hungary, the Netherlands and Switzerland	No impact	No impact
D614G	Substitution (spike): D614G	Rapid increase during the early stages of the pandemic in the EU/EEA and then worldwide, probably due to a mix of founder effects and increased transmissibility	Worldwide. All other variants described here have descended from this one	No impact	No impact

EU/EEA, European Union/European Economic Area.
Table adapted from: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-risk-related-to-spread-of-new-SARS-CoV-2-variants-EU-EEA.pdf.

Following our previous methodology (Penarrubia et al., 2020), we re-evaluated the impact on sensitivity of the mutations of SARS-CoV-2 variants under investigation according to the European Centre for Disease Prevention and Control (2021) (Table 1) for both the QIAstat-Dx Respiratory SARS-CoV-2 panel and the NeuMoDx SARS-CoV-2 assay (Qiagen; https://www.neumodx.com/sars-cov-2/). Both of these assays use a dual target approach; the QIAstat-Dx-Respiratory panel targets the *RdRp* gene and the *E* gene, and the NeuMoDx SARS-CoV-2 assay targets the *Nsp2* gene and the *N* gene. The analysis found that the mutations present in the listed viral strains are not expected to affect the sensitivity and/or inclusivity of the QIAstat-Dx Respiratory SARS-CoV-2 panel or the NeuMoDx SARS-CoV-2 assay.

The SARS-CoV-2 mutation rate will continue to increase the number of variants. Although reports of increased infectivity have appeared, no effect on the virulence of COVID-19 has been reported to date. Here, our results confirm the findings of previous work showing that multi-target real-time RT-PCR SARS-CoV-2 detection can mitigate the risk of loss of sensitivity. In this regard, continuous monitoring of genomic variants by assay manufacturers is essential to provide a rapid response in case the need arises for assay redesign.

Conflict of interests

All authors are employees of QIAGEN.

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Ethical approval

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