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Short communication

# Utility of a commercial RT-qPCR assay to detect SARS-CoV-2 gene variations as an indicator of lineages

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ABSTRACT

*Background:* The World Health Organization (WHO) recommended RT-qPCR tests as the reference technique for SARS-CoV-2 molecular detection, however with the rapid spread of the infection, mutations in specific RT-qPCR target regions have been widely described could allow the presumptive identification.
*Objective:* In this study, we evaluated the analytical performance of the Allplex™SARS-CoV-2/FluA/FluB/RSV assay for the additional presumptive identification of SARS-CoV-2 variants in a real-life setting.
*Results:* We observed gene-specific changes in the cycle threshold (Ct) of the *N* and *RdRp* genes compared with the Ct yielded for the *S* gene when the SARS-CoV-2 testing was performed Allplex™SARS-CoV-2/FluA/FluB/RSV assay.
Seventeen samples showed Ct variations in the *N* and/or *RdRp*. In 10 cases, the *N* gene was affected, delayed or negative and in 14 cases, the *RdRp* gene showed a delay or negative concerning the *S* gene. A delay in the Ct of both genes (*RdRp* and *N*) was observed in six cases. Sequencing determined that all samples identified as B.1.1.7 showed changes in the PCR curves of the *N* and *RdRp*. However, samples identified as B.1.177 only showed variations for the *RdRp* gene.
*Conclusions:* Allplex™SARS-CoV-2/FluA/FluB/RSV assay, the diagnosis could presumably allow the rapid assignment of lineages B.1.1.7 and B.1.177, and emphasizes the importance of exhaustive surveillance for circulating variants of the SARS-CoV-2 virus to reduce community transmission.

## 1. Introduction

In December 2019, the first cases of a new respiratory severe disease were detected in Wuhan, China. The novel β-coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified as the cause of the coronavirus disease 2019 (COVID-19) (Zhu et al., 2020).

At the beginning of the 2020 summer, the B.1.177 lineage was detected in Spain, and few weeks, it became the dominant lineage (González-Candelas et al., 2021). In the second half of December 2020, a new variant from the United Kingdom (UK) denominated B.1.1.7 began to predominate and is currently considered a variant of concern (VOC). It is characterized by a significantly increased transmissibility, which has contributed to increases in incidence, hospitalizations and pressure on the healthcare system (European Centre for Disease Prevention and Control, 2021).

Since the onset of the COVID-19 pandemic, the World Health Organization (WHO) recommended real-time reverse transcription PCR (RT-qPCR) tests as the reference technique for SARS-CoV-2 molecular detection (Azzi et al., 2020; Rao et al., 2021; World Heath Organization (WHO), 2021). Most commercially available assays for SARS-CoV-2 RNA detection target different regions of the viral genome, such as the envelope gene (*E*), the RNA-dependent RNA polymerase gene (*RdRp*), open reading frame 1a and 1b (*ORF1ab*) and the nucleocapsid gene (*N*) (Rao et al., 2021). However, with the rapid spread of the infection, mutations in these specific target regions have been widely described, which may presumably compromise the sensitivity and specificity of these commercial assays in the short term (Hasan et al., 2021; Artesi et al., 2021; Ziegler et al., 2020). Furthermore, different authors have

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suggested that specific mutations among these targeted genes in SARS-CoV-2 could allow the presumptive identification of specific variants using RT-qPCR diagnostic assays (Wollschlaeger et al., 2021).

Herein, we evaluated the analytical performance of the Allplex™SARS-CoV-2/FluA/FluB/RSV assay (Seegene, South Korea) for the additional presumptive identification of SARS-CoV-2 variants in a real-life setting.

## 2. Methods

The Microbiology Department of the Complejo Hospitalario de Navarra, located in Pamplona, northern Spain, is the reference laboratory of the public health system for SARS-CoV-2 testing. In this regard, upper respiratory specimens are routinely collected at the hospital and primary care centres and processed by different RT-qPCR methods. Viral RNA extraction was performed using the viral DNA/RNA extraction cartridge kit STARMag 96 × 4 (Seegene, South Korea) and the Hamilton Microlab STARlet automation robot (Hamilton Company, USA). Since late November 2020, the RT-qPCR Allplex™ SARS-CoV-2/FluA/FluB/RSV assay was implemented for the molecular diagnosis of COVID-19. All technical procedures are performed as described by the manufacturers. Additionally, especially since the emergence of SARS-CoV-2 variant B.1.1.7 in our settings in late December 2020, our laboratory has been performing prospective surveillance of SARS-CoV-2 genetic variants using next-generation sequencing (NGS) approaches.

NGS was performed by the SeqCOVID Consortium (Valencia, Spain) and in the public company NASERTIC (Navarra, Spain) to determine possible mutations, and to identify which variants represent to those samples showing changes in the genes studied in order to explain the RT-qPCR results. Sequences were uploaded to Global Initiative on Sharing Avian Influenza Data (GISAID) (https://www.gisaid.org/) database.

## 3. Results

Between 24 January and 25 February 2021, 52,774 samples were tested for SARS-CoV-2. Of them, 3,190 (6.04 %) resulted positive. Of total positive samples, 762 samples were sequenced, 386 (50.7 %) correspond to lineage B.1.177, 268 (35.2 %) lineage B.1.1.7, and 108 (14.2 %) other lineages. During the routine, we observed gene-specific changes in the cycle threshold (Ct) of the *N* and *RdRp* genes compared with the Ct yielded for the *S* gene when the SARS-CoV-2 testing was performed Allplex™SARS-CoV-2/FluA/FluB/RSV assay.

Seventeen samples showed Ct variations in the *N* and/or *RdRp*. In three cases, the *N* gene was affected, delayed or negative concerning the *S* gene. In eight cases, the *RdRp* gene showed a delay or negative concerning the *S* gene. A delay in the Ct of both genes (*RdRp* and *N*) was observed in six cases. In most cases with Ct delayed in *RdRp* or *N* genes, RT-qPCR Ct values were > 35, and the difference between Ct was >5 Ct (Table 1).

Sequencing in these cases determined that samples tested by Allplex™SARS-CoV-2/FluA/FluB/RSV assay showed in the presence of the lineage B.1.1.7, the *N* gene was delayed or not amplified, and in six of the nine cases sequenced as B.1.1.7 the *RdRp* gene was also delayed concerning the *S* gene. However, samples of the lineage B.1.177 showed only a delay or negativity of the *RdRp* gene.

## 4. Discussion

Our results determined that all samples identified as B.1.1.7 showed changes in the PCR curves of the *N* gene and in some cases also the *RdRp* gene when the Allplex™SARS-CoV-2/FluA/FluB/RSV assay was used. However, samples identified as B.1.177 only showed variations for the *RdRp* gene. These results suggest the possibility for a pre-screening of these lineages.

The *N* gene results obtained using the same RT-qPCR assay (Allplex™SARS-CoV-2/FluA/FluB/RSV) concord with those published by Wollschlaeger and coworkers recently where 48 samples belonged to the B.1.1.7 lineage showed Ct values delayed or negative values of the *N* gene. In contrast, the authors did not observe changes related to the *RdRp* gene (Wollschlaeger et al., 2021). On 23 February 2021, during the manuscript writing, Seegene Inc. published a confirmation letter assessing that in the presence of the variant B.1.1.7, Allplex SARS-CoV.-2/FluA/FluB/RSV assay showed the shape of the raw curve of the channel corresponding to *RdRp/S* gene differed from the expected shape of the curve. Besides, the Ct value of the channel corresponding to *N* gene tended to appear delayed. This publication suggests the possibility to use this kit as a pre-screening tool to detect this lineage. According to this publication, the delayed Ct values detected in this study to both genes (*RdRP/S* and *N*) could mean the presence of the B.1.1.7 lineage.

**Table 1**
RT-qPCR Ct values observed among the 17 study samples with the corresponding lineage.

| Patient | RT-qPCR (Allplex™ SARS-CoV-2/FluA/FluB/RSV) | | | Lineage |
|---|---|---|---|---|
| | *S* | *RdRp* | *N* | |
| 1 | 15.63 | 22.45 | 37.32 | B.1.1.7 |
| 2 | 14.5 | 21.60 | 36.19 | B.1.1.7 |
| 3 | 11.52 | 18.90 | 27.28 | B.1.1.7 |
| 4 | 25.00 | 33.26 | 38.52 | B.1.1.7 |
| 5 | 18.00 | 26.96 | 39.48 | B.1.1.7 |
| 6 | 26.00 | 26.00 | 35.36 | B.1.1.7 |
| 7 | 22.10 | 25.23 | 33.17 | B.1.1.7 |
| 8 | 23.14 | 29.56 | N/A | B.1.1.7 |
| 9 | 30.00 | 31.22 | N/A | B.1.1.7 |
| 10 | 28.48 | 36.23 | 28.43 | B.1.177 |
| 11 | 29.35 | 34.54 | 28.67 | B.1.177 |
| 12 | 29.36 | 39.15 | 29.15 | B.1.177 |
| 13 | 24.36 | 29.20 | 22.80 | B.1.177 |
| 14 | 28.35 | 37.92 | 28.22 | B.1.177 |
| 15 | 28.41 | 35.10 | 29.83 | B.1.177 |
| 16 | 22.80 | N/A | 21.50 | B.1.177 |
| 17 | 21.30 | N/A | 24.82 | B.1.177 |

N/A: not amplified.

Furthermore, other studies have also described gene delays using other RT-qPCR assays (Hasan et al., 2021; Artesi et al., 2021; Ziegler et al., 2020; Vanaerschot et al., 2020). Using the Cepheid GeneXpert system, Hasan and coworkers reported a novel *N* gene mutation (C29200A), which affected the detection of the SARS-CoV-2 *N* gene by a commercial assay. In our study, we did not describe new mutations for the *RdRp* and *N* genes that could affect the detection of the virus using Allplex™SARS-CoV-2/FluA/FluB/RSV. However, it would be highly recommended to carry out a more exhaustive study to conclude the presence of possible mutations that could interfere with technique's sensitivity.

The present study has some limitations. Although variations in *N* and *RdRp* genes were detectable as B.1.1.7 and changes only in the *RdRp* gene were B.1.177, the sample size was small and it is not possible to be sure exactly what other variables may exert pressure on the diagnosis. Furthermore, it must be considered that mutations in these genes can appear in other variants.

In conclusion, Allplex™SARS-CoV-2/FluA/FluB/RSV assay, in addition to SARS-CoV-2, Influenza A and B and RSV, the diagnosis could presumably allow the rapid assignment of lineages B.1.1.7 and B.1.177, and emphasizes the importance of exhaustive surveillance for circulating variants of the SARS-CoV-2 virus to reduce community transmission.

## Data availability

No data was used for the research described in the article.
All Data will be made available on request.
The data that has been used is confidential.

## Author statement

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## Availability of data and material

All genomes generated in this work were deposited in the GISAID database (http://gisaid.org).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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