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Short Communication

Rapid screening for SARS-CoV-2 VOC-Alpha (202012/01, B.1.1.7) using the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay

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ABSTRACT

Background: The emergence of SARS-CoV-2 variants of concern (VOCs) for increased transmissibility and being potentially capable of immune-escape mandates for epidemiological surveillance. Genomic alterations present in VOCs can affect the results of RT-qPCR assays for routine diagnostic purposes, leading to peculiar profiles that can be used for rapid screening of variants. This study reports a peculiar profile observed with the Allplex™ SARS-CoV-2/FluA/FluB/RSV assay and VOC-Alpha (202012/01, lineage B.1.1.7, also named VOC-UK), which was the first identified SARS-CoV-2 VOC.

Methods: Samples were analyzed by two RT-qPCR assays: the Allplex™ SARS-CoV-2/FluA/FluB/RSV assay (ASFR, Seegene Technologies Inc; Seoul, South Korea) and the TaqPath COVID-19 RT-PCR (Thermo Fisher Scientific, USA). Definition of the SARS-CoV-2 variant was carried out by Sanger sequencing of the relevant S-gene regions and, in some cases, by whole genome sequencing (WGS) using the ARTIC-nCoV workflow on a MiniION (Oxford Nanopore Technologies, Oxford, UK) or a Illumina MiSeq platform (San Diego, California, USA).

Results: Of the 173 SARS-CoV-2-positive specimens, all those of lineage B.1.1.7 (N=71) showed an average Cq difference between the N and S genes of +11±2 (range, +8/+15). None of the other specimens, including several different lineages (Wild-type for the analyzed regions, N=22; Gamma, N=63; Delta, N=9; B.1.258Δ, N=3; B.1.160, N=3; B.1.177.7, N=1; B.1.1.420, N=1), exhibited a similar difference in Cq values.

Conclusions: The peculiar pattern of delayed N gene positivity could constitute a convenient method for VOC-Alpha screening, simultaneous to viral detection, when using the Allplex™ SARS-CoV-2/FluA/FluB/RSV assay.

The current SARS-CoV-2 pandemic has crucially affected populations and healthcare systems worldwide. One of the challenges posed by SARS-CoV-2, similar to other RNA viruses, is represented by genomic variability, which may lead to the emergence of variants with improved transmissibility, increased virulence and/or immune-escape ability. Variants for which clear evidence is available about such modified features, which are likely to have an epidemiological impact, have been categorized as variants of concern (VOCs) (ECDC, 2020). Among them, VOC-Alpha (202012/01, belonging to the B.1.1.7 lineage, also named VOC-UK) was the first to be identified. Indeed, this variant rapidly disseminated worldwide, becoming the dominant circulating lineage in several areas, due to its higher transmissibility (Leung *et al.*, 2021). VOC-Alpha is defined by multiple spike protein alterations, including His69-Val70 and Tyr144 deletions, and amino acid substitutions Asn501Tyr, Ala570Asp, as well as alterations in other proteins (e.g. Asp3Leu and Ser235Phe in the N nucleocapsid protein) (Haynes *et al.*, 2021).

The emergence and spread of SARS-CoV-2 variants has mandated for their epidemiological surveillance. Although sequencing the entire viral genome represents the reference approach for variant identification, it is expensive and time-consuming and requires specialized laboratories. Therefore, there is considerable interest for rapid molecular methods able to screen for VOCs, which could be helpful to rapidly detect their presence by routine lab-

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Table 1
Summary of the results obtained from 173 specimens positive for SARS-CoV-2, analyzed with the AFSR and TaqPath COVID-19 RT-PCR assays.

	ASFR		TaqPath	
Lineage	N. of specimens	Average ΔCq (N-S genes)	Cq range	Missing S-gene target amplification
Alfa (B.1.1.7)	71[a]	+11	from +8 to +15	71
Gamma (P1)	63[b]	+1	from -2 to +5	0
Delta (B.1.617.2)	9[c]	+4	from +1 to +6	0
B.1.258Δ	3[c]	-2	from -4 to 0	3
B.1.160	3[c]	0	from -1 to 0	0
B.1.177.7	1[c]	-1	-1	0
B.1.1.420	1[c]	-1	-1	0
Wild-type*	22	-1	from -3 to +1	0

* Wild-type for the analyzed regions by Sanger sequencing
[a] five of them confirmed by WGS
[b] one of them confirmed by WGS
[c] all confirmed by WGS

oratory diagnostics and timely implementation of tailored contact tracing and restriction measures. For instance, the TaqPath COVID-19 RT-PCR (Thermo Fisher Scientific, USA), which fails amplification of the S gene target in case of the 69-70 deletion typical of VOC-Alpha, has successfully been used for rapid screening of this VOC based on such a peculiar amplification profile (Kidd *et al.*, 2021).

This study reports the observation that VOC-UK could be easily and rapidly detected by analyzing the profile of results obtained by the Allplex™ SARS-CoV-2/FluA/FluB/RSV assay (ASFR, Seegene Technologies Inc; Seoul, South Korea). ASFR is a multiplex real-time RT-PCR assay targeting the N, RdRP and S genes of SARS-CoV-2, along with influenza A, influenza B and respiratory syncytial virus (RSV). A collection of 173 SARS-CoV-2-positive, non-replicate, nasopharyngeal swabs were evaluated. All samples, residual from the diagnostic routine, were anonymized for the purpose of this study. The SARS-CoV-2 lineage was presumptively assigned by characterization of two regions of the S gene (21663-22036 bp and 22772-23460 bp) by Sanger sequencing. Twenty-three specimens were also subjected to whole genome sequencing (WGS) by the ARTIC-nCoV workflow using a MiniION platform (Oxford Nanopore Technologies, Oxford, UK) (Tyson *et al.*, 2020) or by the EasySeq RC-PCR SARS-CoV-2 Whole Genome Sequencing kit (NimaGen BV, Nijmegen, NL) on an Illumina MiSeq platform (San Diego, USA). Overall, the collection included 71 specimens (of which five were confirmed by WGS) of the B.1.1.7 lineage, 63 specimens (of which one was confirmed by WGS) of the P.1 lineage, nine specimens of the Delta (B.1.617.2) lineage (all confirmed by WGS), and 30 specimens belonging to other lineages not currently included among VOCs (of which eight were confirmed by WGS) (Table 1).

In addition to the ASFR assay, all specimens were also analyzed with the TaqPath COVID-19 RT-PCR, which has been used for rapid screening of VOC-UK (Kidd *et al.*, 2021). Analysis of the results obtained with specimens of the B.1.1.7 lineage always revealed a notable difference between the Cq values for the N and S genes (mean, +11±2; range, +8/+15), while such a difference was not observed with specimens of the other lineages (Table 1). The TaqPath COVID-19 RT-PCR assay revealed a missing S-gene target amplification with all specimens of VOC-UK, but also with B.1.258Δ (Table 1), as expected from the presence of the HV69-70 spike deletion also in members of this lineage (Brejová *et al.*, 2021).

The delayed detection of the N-gene target (i.e. higher Cq values) with the ASFR assay might be due to the presence of mutations 28280 GAT->CTA and C28977T in the N nucleocapsid gene, likely affecting the oligonucleotide/probe region and leading to lower RT-qPCR efficiency.

In conclusion, these data suggest that finding a considerably higher (>8) N-gene Cq value *vs.* S-gene Cq value could be highly suggestive of the presence of VOC-UK at the same time as viral detection. The delayed N-gene target amplification by the ASFR assay may constitute a more specific proxy for VOC-UK compared with the TaqPath test, which presumptively identifies VOC-UK based on a missing S-gene target amplification due to the presence of HV69-70 deletion, which is also present in other lineages such as B.1.258Δ. In fact, members of the latter lineage did not present a delayed N-gene amplification with the ASFR assay (Table 1).

Further experiments, including additional variants with alterations in the N-gene sequence, will be required to confirm the specificity of the ASFR "N-late" method for rapid screening of VOC-UK. Similar approaches, based on modified patterns of results obtained with commercial diagnostic resources routinely used for SARS-CoV-2 detection, could be of interest for inexpensive and large-scale epidemiological surveillance of viral variants.

DECLARATION OF COMPETING INTEREST

Dr. Antonelli reports personal fees from Accelerate diagnostics, personal fees from Arrow diagnostics, personal fees from Menarini, personal fees from Seegene, non-financial support from SymCel, outside the submitted work.

Dr. Rossolini reports grants, personal fees and non-financial support from Accelerate Diagnostics, personal fees from Becton Dickinson, grants and personal fees from bioMérieux, grants and personal fees from Cepheid, grants and personal fees from Elitech, grants and personal fees from Merck, grants and personal fees from Nordic Pharma, personal fees from Pfizer, grants from Seegene, grants and personal fees from Shionogi, personal fees and other from Venatorx, grants and personal fees from Zambon, personal fees from Roche, personal fees from Thermo Fisher, personal fees and non-financial support from Beckman Coulter, grants, personal fees and non-financial support from Menarini, grants from Arrow, grants from Symcel, personal fees from QPex, grants from DID, grants from Hain Lifescience GmbH, grants from GenePoc, grants from SetLance, grants and personal fees from Angelini, grants from Qvella, grants from Qlinea, personal fees from Qiagen, grants from Biomedical Service, grants from Liofilchem, outside the submitted work.

Dr. Baccani reports for Diesse-Diagnostica Senese, outside the submitted work.

All other authors have nothing to disclose.

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AUTHOR STATEMENT

NG wrote the original draft, which was reviewed and edited by GMR, MC, AA, FM and SP. The study was conceived by NG, GMR, AA and MC; investigation was performed by NG, NA and IB; resources were accessed by GMR; NG, NA, IB, MC and AA contributed to the methodology; NG, MC and AA contributed to the formal analysis; project administration was by GMR and SP.

ETHICAL APPROVAL STATEMENT

Results were obtained from the analysis of anonymized data derived from diagnostic routine testing.

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