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Reduced amplification efficiency of the RNA-dependent-RNA-polymerase target enables tracking of the Delta SARS-CoV-2 variant using routine diagnostic tests

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ABSTRACT

Routine SARS-CoV-2 surveillance in the Western Cape region of South Africa (January-August 2021) found a reduced RT-PCR amplification efficiency of the RdRp-gene target of the Seegene, Allplex 2019-nCoV diagnostic assay from June 2021 when detecting the Delta variant. We investigated whether the reduced amplification efficiency denoted by an increased RT-PCR cycle threshold value (RΔE) can be used as an indirect measure of SARS-CoV-2 Delta variant prevalence. We found a significant increase in the median RΔE for patient samples tested from June 2021, which coincided with the emergence of the SARS-CoV-2 Delta variant within our sample set. Whole genome sequencing on a subset of patient samples identified a highly conserved G15451A, non-synonymous mutation exclusively within the RdRp gene of Delta variants, which may cause reduced RT-PCR amplification efficiency. While whole genome sequencing plays an important in identifying novel SARS-CoV-2 variants, monitoring RΔE value can serve as a useful surrogate for rapid tracking of Delta variant prevalence.

Genomic surveillance of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by whole genome sequencing (WGS) has played a critical role in identifying and monitoring the dissemination of variants of concern (VOCs) (Abdool Karim and de Oliveira, 2021; WHO, 2021). However, WGS is costly and time-consuming, and not all countries have the resources to do this at scale to get detailed VOC epidemiological information.

Multiplex reverse transcriptase polymerase chain reaction (RT-PCR)-

Abbreviations: SARS-CoV-2, Severe Acute respiratory Syndrome Coronavirus 2; WGS, Whole Genome Sequencing; VOCs, Variants of Concern; S, Spike; N, Nucleocapsid; E, Envelope; RdRp, RNA-dependent-RNA-polymerase; NHLS, National Health Laboratory Service; RΔE, difference between the Ct values for RdRp and E-gene targets; RT-PCR, reverse transcriptase polymerase chain reaction; NGS-SA, Network for Genomic Surveillance South Africa; GISAID, Global Initiative on Sharing All Influenza Data; AUC, Area Under the Curve; GTF, Gene Target Failure; IQR, Interquartile range.

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based diagnostic tests for SARS-CoV-2, which simultaneously amplify and detect multiple SARS-CoV-2 gene targets, such as the Spike (S)-, Nucleocapsid (N)-, Envelope (E)- and RNA-dependent-RNA-polymerase (RdRp) genes, are the primary tools used to diagnose cases and define epidemic waves. Current commercial tests were designed to detect ancestral SARS-CoV-2 sequences and have not been optimised for the viral genomic diversity that has subsequently emerged (WHO, 2020). Despite this, built-in redundancy through detecting multiple gene targets ensures that these assays continue to be effective tools for diagnosing infections by contemporary variants as diagnostic assays do not need to detect all target genes. For example, deletions of amino acids 69 and 70 within the S-gene of SARS-CoV-2 Alpha and Omicron variants was associated with failure to detect this target by some commonly used commercial diagnostic assays (e.g. ThermoFisher TaqPath, Waltham, USA). Fortuitously, because the other targets are largely unaffected, this S-gene target failure (S-GTF) has been used as a convenient surrogate marker for monitoring prevalence of SARS-CoV-2 Alpha variant (Brown et al., 2021; Volz et al., 2021).

As the Delta variant becomes the predominant VOC across the world, a similar diagnostic RT-PCR-based marker has potential to enable similar rapid epidemiological assessment and research. An evaluation of routine diagnostic results together with WGS, we show that infection with the Delta variant is associated with reduced RdRp target amplification efficiency and propose that this can be used for tracking the prevalence of this variant.

Routine SARS-CoV-2 diagnostic testing in the South African public health sector, which is used by circa 80 % of the population is performed by the National Health Laboratory Service (NHLS). NHLS laboratories also form part of the network for genomic surveillance South Africa (NGS-SA) where a subset of routine diagnostic samples are sequenced as part of the genomic surveillance activities (Msomi et al., 2020). WGS was performed using either GridION X5 (Oxford Nanopore Technologies, Oxford, United Kingdom) or MiSeq (Illumina, San Diego, USA) sequencing platforms (Tegally et al., 2021). To ensure accurate clade assignment, we only included sequences with less than 3000 bases missing, no frameshift mutations and no misplaced stop codons. All sequences were deposited on the Global Initiative on Sharing All Influenza Data (GISAID) SARS-CoV-2 sequence repository (https://www.gisaid.org/) (Supplementary Table 1).

Between March 2020 and August 2021, the Seegene Allplex 2019-nCoV assay (Seoul, South Korea), a single-tube multiplex assay with three SARS-CoV-2 gene targets: E, RdRp and N, was one of the most commonly used RT-PCR platforms across the NHLS. From May 2021, we observed a consistent increase in the number of samples testing positive with the Seegene Allplex nCoV-19 assay that had either delayed or absent RdRp target detection (cycle threshold (Ct) value) relative to E gene and N gene targets. Prior to this period, the average difference between the Ct values for RdRp and E-gene targets (RΔE) was around 2. From June 2021, the RΔE in diagnostic samples increased to a median of 5 (Fig. 1). We investigated if the efficiency of RdRp target amplification and detection was affected by mutations in the predominant circulating variant.

The Western Cape province where the study was conducted, entered its third epidemic wave on 21 June 2021 (week 25), with more than 1020 cases reported daily and >20 % week-on-week increase in the 7-day moving average of new cases. During the same period, genomic surveillance data showed a rapid increase in Delta variant infection, which rapidly replaced Beta as the predominant VOC in the region (Fig. 2). As the cases surged, the proportion of Delta variant increased from just over 20 % of total samples sequenced in May 2021, to >90 % in August 2021.

To investigate the observed RdRp target delay which coincided with the emergence of Delta, RdRp gene sequences of samples tested with the Seegene, Allplex 2019-nCoV assay were evaluated for mutations that could interfere with RT-PCR probe or primer binding. We identified a non-synonymous G15451A mutation (codon 671S) within RdRp gene of

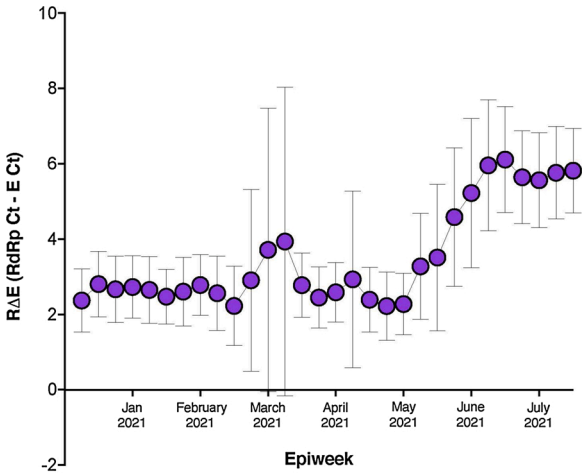

Fig. 1. Mean difference between RdRp and E-gene Ct values for samples tested by the Seegene Allplex 2019-nCoV assay (ΔCt). The mean ΔCt is shown for samples testing positive for both E and RdRp targets for each week of 2021 up to 31 July. Only samples tested in the Western Cape region of South Africa are included in the analysis. Error bars represent 1 standard deviation.

Delta variants, potentially responsible for the reduced amplification efficiency of the assay RdRp target. This highly conserved G15451A mutation was present in 100 % (369/369) of Delta variant sequences and not observed in Alpha (n = 11), Beta (n = 749) or Eta (n = 5) variants sequenced between March 2020 and 19 August 2021 (Supplementary Fig. 1). This specific mutation was significant as it resulted in a single nucleotide mismatch in the second last base of the WHO-recommended forward primer binding site for RdRp amplification for most diagnostic assays targeting RdRP, including Seegene Allplex nCoV-19 (Corman et al., 2020). In addition, we previously found that a synonymous G15452C mutation (codon 671S) in a minority of Beta variant sequences (3′-end of this forward primer binding site) also affected RdRp gene target detection when using the Seegene, Allplex 2019-nCoV assay (Supplementary Fig. 1).

To systematically assess the effect of this Delta variant G15451A mutation on Seegene assay RdRp target amplification efficiency relative to that of ancestral strain and Beta variant, we compared the median relative RdRp and E gene target Ct values (RΔE) among the different World Health Organisation SARS-CoV-2 designated variants detected in diagnostic samples over this time period. Of the 1455 sequences retrieved, 39 sequences with incomplete Ct data was removed, leaving 9, 731, 362 and 314 were Alpha, Beta, Delta and other variants respectively. Delta variant samples had a significantly increased RΔE (n = 362, median = 5.74, Interquartile range (IQR) 4.76–6.55) when compared to non-Delta variant samples (n = 1056, median = 2.54, IQR 2.13–3, $p < 0.001$) (Supplementary Fig. 2). The diagnostic ability of the RΔE value to identify the Delta variant was evaluated using a receiver operating characteristic (ROC) curve (Supplementary Fig. 3). Using a RΔE threshold of > 3.5 cycles we were able to accurately identify Delta variant positive samples within the sample set with 93.6 % sensitivity and 89.7 % specificity, correctly classifying 90 % of cases (Area under the curve (AUC) = 0.9663). When the threshold is increased to 4 and 4.5 cycles, the specificity was improved to 96.5 % and 98 % respectively but the sensitivity was reduced to 86.7 % and 80 % respectively.

In this article we show that reduced amplification efficiency of the RdRp gene target of the Seegene, Allplex 2019-nCoV assay can be used as an indirect measure of SARS-CoV-2 Delta variant prevalence in a population. Using the RΔE value could therefore serve as a reliable surrogate for genomic sequencing to approximate the spread of the SARS-CoV-2 Delta variant. While using the RΔE is only a surrogate marker of Delta variant identity, assay Ct values have proven useful in the past to identify Alpha variant prevalence as well as serving as an

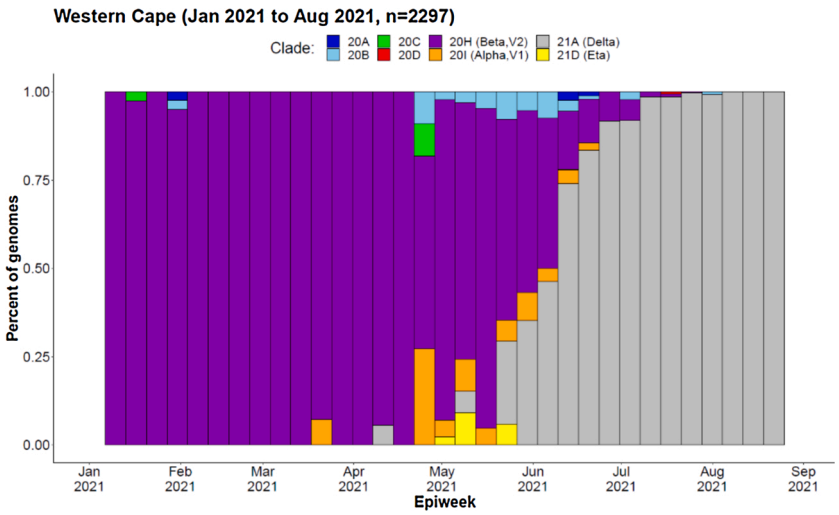

Fig. 2. Weekly frequency and distribution of SARS-CoV-2 variants circulating in the Western Cape region of South Africa between 1 January and 31 August 2021.

independent predictor of disease severity (Yu et al., 2020; Choudhuri et al., 2020). Limitations to our study concern the proprietary nature of the Seegene, Allplex 2019-nCoV assay. We are unable to confirm the exact genomic loci amplified by the assay primers and probes. Evidence including the RdRp target delay in Delta variants from this study, RdRp complete GTF in a minority of Beta variants and confirmation by Seegene that the G15451A mutation in Delta variants leads to a mismatch in the primer binding site, suggest that these diagnostic primers overlap with the WHO-recommended RT-PCR primer sets stemming from Corman et al., 2020 (Corman et al., 2020). This study highlights the need for continued monitoring of the efficacy of current commercial SARS-CoV-2 diagnostic assays in a setting where we are observing the constant genetic drift of a novel pathogen.

Author statement

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Disclaimers

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the funding agencies.

Data availability

Data will be made available on request.

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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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2022.114471.

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