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# Fast and cost-effective screening for SARS-CoV-2 variants in a routine diagnostic setting

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## ABSTRACT

**Objective.** The aim is to recommend a fast and cost-effective screening procedure for UK/SA SARS-CoV-2 variants in a routine diagnostic setting.

**Methods.** A rapid procedure using qPCR is described to provide clinicians with information about the two currently most prevalent variants (B.1.1.7 and B.1.351) that harbour receptor binding domain mutation N501Y. The N501Y specific assay only delivers an amplification signal if the Y501 variant is present.

**Results.** 436 samples initially screened positive for SARS-CoV-2 were randomly selected. Only one of these samples showed a fluorescence signal increase indicative for the Y501 variant. The remaining 435 samples had a melting peak at 54 °C indicating the N501 wildtype.

**Significance.** The screening of a broad population base can still be performed with the established test system. In case of a positive test for SARS-CoV-2 and corresponding clinical and anamnestic indications, a second qPCR for the mutation N501Y can follow and deliver the result to public health authorities and to the treating physician within a few hours.

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## 1. Introduction

The rapid evolution of viruses is a key to their success. A factor influencing the mutation rate and thereby evolution is, among others, the type of genetic material. RNA viruses usually mutate faster than DNA viruses, single-strain viruses faster than double-strain viruses [1]. Mutations can result in changes at multiple levels of viral replication. An easier entry into the cell, release of infectious particles, drug resistance,

escape from the immune system response, the transition to other species is also possible based on the mutations, to name a few points [1,2]. In the context of a pandemic, these mutations are of particular importance, especially if they increase the severity of the disease as currently described for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus [3].

Therefore it is crucial to continuously monitor the circulating viral population in order to detect new variants as soon as possible.

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Sequencing is the basis of detection of novel variants and has to be performed from a representative portion of positive samples. The Covid-19 genomics UK consortium ([www.cogconsortium.uk](http://www.cogconsortium.uk)) has sequenced over 200,000 viruses up to date (January 18th 2021), thus allowing the identification of the recently described highly contagious variant B.1.1.7. and the similar South African variant B.1.351.

In an event like the current SARS-CoV-2 pandemic, many people are affected, who have to be diagnosed and treated. The confirmed pathogen detection should be available within a few hours so that appropriate clinical decisions can be made and those affected can be helped.

Mutations are not only decisive for the clinical course, but the knowledge of the circulating variants might provide valuable information for public health authorities to decide further measurements to limit infection rates (e.g. more extensive lockdown).

This also poses a challenge for the laboratory. On the one hand, testing must be available to a broad base, and on the other hand, mutations must also be available through complex and time-consuming sequencing in order to ensure optimal care.

Sequencing by means of next generation sequencing (NGS) is being carried out more and more frequently in laboratories and is not widely available. Sequencing, including sample preparation, at least takes 3–4 days, not including shipping to a center [4].

However, using sequencing to detect already known variants within a viral population might be a waste of time and of valuable resources.

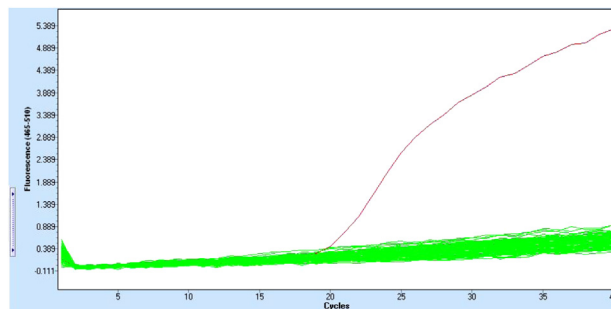
In the current situation, many laboratories are performing several hundred to a thousand tests, some in the dual-target strategy, for the SARS-CoV-2 virus. The current mutants from the UK and South Africa are recognized as SARS-CoV-2 virus, but not as mutants.

However, for many clinical questions and especially for public health authorities, it is important to know if this mutant is present. However, sequencing every positive case is neither possible, nor necessary, nor practicable. Therefore, we report on a solution using qPCR that quickly provides clinicians with the information about the two mutants and does not burden the sequencing capacity with unnecessary samples, but reserves it for a few individual cases. Both the SARS-CoV-2 variant from South Africa and the UK harbor the receptor binding domain mutation N501Y, a spike protein that allows the virus to enter human cells [3].

This mutation is not present in other, widely circulating strains. We took advantage of this circumstance to introduce qPCR for specifically this mutation into routine diagnostics. The lack of detection of the N501Y mutation in qPCR accordingly excludes the South African and UK variants quickly and does not require sequencing.

## 2. Material and methods

Nasal and throat swabs that are transported in 0.9% NaCl as transport medium to the laboratory were used. A 200  $\mu$ l aliquot of the sample was transferred into a cavity of a 96-well plate. SARS-CoV-2 RNA extracted with MagNA Pure 96 (Roche



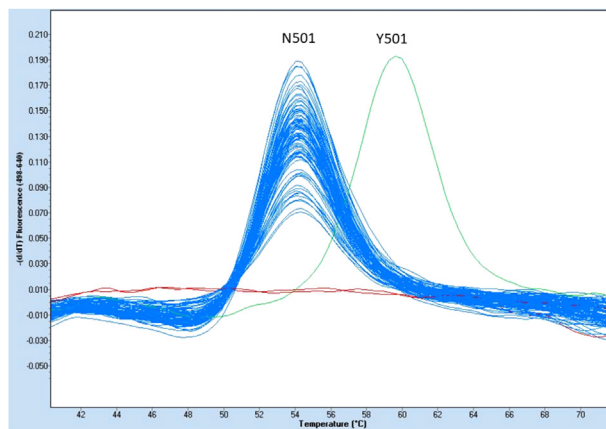
**Fig. 1 – qPCR from a 96 samples run including two SARS-CoV-2 negative samples and one Y501 variant. Only the Y501 variant showed an increase of the fluorescence signal.**

Diagnostics, Penzberg, Germany) and using the viral NA small volume kit (Roche Diagnostics). The elution was done in 50  $\mu$ l. 5  $\mu$ l of the extracted RNA used in a qPCR in total of 20  $\mu$ l using the VirSniP SARS-CoV-2 Spike A23063T N501Y (TIB-Molbiol, Berlin, Germany) assay with the RNA Process Control Kit (Roche Diagnostics) according to manufacturers' instruction. The N501Y specific assay delivers only an amplification signal if the Y501 variant is present. Subsequent melting analyses were done in a LightCycler<sup>®</sup> 480 II (Roche Diagnostics), to differentiate between the N501, Y501 variants and negative samples.

## 3. Results

436 samples initially screened positive for SARS-CoV-2 using the RIDA<sup>®</sup> GENE SARS-CoV-2 assay (r-Biopharm, Darmstadt, Germany), were randomly selected. The samples came from Munich and southern Bavaria and were analyzed between the December 25th 2020 and January 6th 2021.

Using the N501Y assay, no signal could be detected during qPCR for 435 samples, indicative for wildtype N501. One sample showed an increase of fluorescence signal at 510 nm (Fig. 1), indicative for the Y501 variant.



**Fig. 2 – Melting analysis from a 96 samples run. The Y501 variant showed a higher melting point compared with N501. Two SARS-CoV-2 negative samples did not show any melting peak.**

During melting analysis 2 melting peaks at 54 and 60 °C could be detected, indicating N501 (wildtype) for 435 samples and Y501 variant for 1 sample, respectively (Fig. 2).

The German reference laboratory for corona viruses (Labor Berlin, Charité Vivantes GmbH, Berlin, Germany) confirmed our result for the detected variant (personal communication).

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#### 4. Discussion

Our results demonstrate the feasibility of an approach combining qPCR and melting point analysis for fast and reliable detection of specific corona virus variants. Using this approach we showed that currently, the UK/SA variants seem not to be widely distributed in south Bavaria, thus only accounting for one case out of 436 positives.

A further close monitoring of the epidemiologic situation seems to be highly recommendable.

Thus, the following procedure seems to be reasonable: The screening of a broad population base can still be performed with the established test system. In case of a positive test for SARS-CoV-2 and corresponding clinical and anamnestic indications, a second qPCR for the mutation N501Y can follow

and deliver the result to public health authorities and to the treating physician within a few hours.

If the test for this mutation is negative, but there is clinical evidence of a mutant, e.g., based on the severity of the course, history, sequencing can be initiated.

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