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Short communication

The challenge of screening SARS-CoV-2 variants of concern with RT-qPCR: One variant can hide another

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ABSTRACT

Introduction: Following the emergence of SARS-CoV-2 variants of concern (VOCs) worldwide, it is important to monitor local epidemiology to better understand the occurrence of clusters, reinfections, or infection after vaccination. Detecting mutations by specific RT-qPCR is a rapid and affordable alternative to sequencing. However, care must be taken to ensure that the techniques used are up-to-date and adapted to the variants circulating in the studied population.

Material and methods: All samples tested positive for SARS-CoV-2 were screened for detection of mutations of the spike protein using the Novaplex™ SARS-CoV-2 Variants I Assay from week 11 of 2021. Target sought were deletion H69/V70 and mutations N501Y and E484K. From week 18 we used in addition the new Novaplex™ SARS-CoV-2 Variants II Assay for samples with no targets found with the Variants I assay or with the mutation E484K alone, in order to screen the mutations L452R, K417N/T and W152C.

Results: Between weeks 11 and 25, 2239 positive samples out of 54,317 were tested with the Variants I Assay. Between weeks 18 and 25, 94 samples met the criteria for being tested with the Variants II Assay. Of these, 47 had the L452R mutation without the W152C mutation, typical in the B.1.617 variant. At week 25, this profile was found in 45.5 % of the samples and was the most frequent.

Conclusion: According to our observations, variant B.1.617 has become predominant in our institution and most probably in our region. In the absence of the use of the Variants II Assay, they would have been considered wild.

1. Introduction

For several months now, new variants of the SARS-CoV-2 have been appearing in various geographies (Naveca et al., 2021; World Health Organisation, 2021). Variants of interest (VOIs) and variants of concern (VOCs) have clinical and epidemiological implications. Indeed, mutations found in some VOCs have been associated sometimes with either a decrease in antibody neutralization or greater contagiousness (Jangra et al., 2021; Leung et al., 2021; Wang et al., 2021). This is respectively the case for the E484K mutation, found in the B.1.351 and P.1 variants, and for the N501Y mutation found in these two VOCs as well as in the B.1.1.7 (Grabowski et al., 2021; Munitz et al., 2021; Zhao et al., 2021; Zucman et al., 2021). The challenge for a COVID laboratory is to distinguish these different variants from the wild-type virus Wuhan-Hu-1 in order to better understand local epidemiology, explain possible re-infections or post-vaccination infections, and document clusters. Here we explain how we track the most frequent VOC changes using a RT-qPCR technique and how we discovered that B.1.617 became predominant in our population.

2. Material and methods

2.1. Molecular detection of SARS-CoV-2 with RT-qPCR

In our laboratory, molecular testing for Covid-19 can be done using 3 different methods depending on the workflow (Fig. 1). First-line molecular analyses are performed either by Transcription Mediated Amplification using the Aptima® SARS-CoV-2 assay kit (Hologic, San Diego, CA, USA) on a Panther platform, or by PCR using the Allplex® kit (Seegene

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Technologies, Seoul, South Korea) after extraction with the STARMag Viral DNA/RNA 200 C Kit (Seegene Technologies, Seoul, South Korea) on a STARLet platform (Hamilton Company, Reno, NV, USA) or without any extraction following an internal protocol (Blairon et al., 2021). Results that need to be answered very quickly for emergency reasons (e.g. emergency surgery) are performed by a Loop-Mediated Isothermal Amplification (LAMP) with ID NOW™ COVID-19 (Abbott Molecular Diagnostics, Des Plaines, IL, USA).

2.2. Molecular detection of SARS-CoV-2 mutations with RT-qPCR

Any positive sample has been tested with the Novaplex™ SARS-CoV-2 Variants I Assay from the 11th to the 25th week of 2021. Starting on week 18, all samples tested with the Variants I Assay for which no mutation was detected and those for which only the E484K mutation was found were submitted to the Novaplex™ SARS-CoV-2 Variants II Assay, available since early May. The search for variants with these kits requires extraction according to the same protocol as described above.

3. Results

Between weeks 11 and 25 (March 7 to June 19, 2021), 2239 positive samples (after elimination of duplicates) among a total of 54,317 were tested with the Variants I Assay. Between weeks 18 and 25, 94 samples met the internal criteria for a reflex test with the Variants II Assay. Of these 47 had the L452R mutation. Fig. 2A shows the proportion of the different variants detected with the Variants I Assay, whereas Fig. 2B takes into account the use of the Variant II Assay from week 18 onwards and highlights the supposed variants B.1.167 which would have been considered as a wild strain if only the Variant I Assay had been used. This shows an increase in the number of variants carrying the L452R mutation between weeks 22 and 25. On week 25, this mutation was found in 45.5 % of the overall samples. This variant thus exceeded in number the assumed B.1.1.7 variant (38.6 %), predominant till week 24 (Fig. 2).

4. Discussion

While the reference method for the identification of variants is next generation sequencing (NGS), this method is carried out only in a limited number of centers equipped with the necessary infrastructure (European Centre for Disease Prevention and Control, 2021). Moreover NGS is expensive, slow, and therefore unsuited to mass use. In recent months, we have implemented a RT-qPCR test for the detection of VOCs in routine. Unlike NGS, only some specific mutations of the spike protein of SARS-CoV-2 are amplified. The Novaplex™ SARS-CoV-2 Variants I Assay diagnoses the presence of E484K, N501Y mutations and H69/V70 deletion (Kami et al., 2021). This test is used as a second line test after a positive SARS-COV-2 molecular test has been found. Thanks to this kit, we can inform prescribers of the presumed VOCs associated with a positive test, the absence of mutation detected a priori signing a wild-type virus. However new VOCs have appeared and are spreading. Among these, the Indian variants B.1.617, carrying other mutations than those previously mentioned. Since early May 2021, a new Novaplex™ SARS-CoV-2 Variants II Assay has been available on the market to detect L452R, K417N, K417T and W152C mutations. While, illogically, the proportion of positive samples with no mutation with the kit Variants I was increasing, the implementation in routine of the Variants II Assay was able to demonstrate that the majority of the viruses that were believed to be wild-type were in fact VOCs of another type. In this study, we draw the attention of the users of the Variants I Assay to the fact that its use alone can be the cause of misinterpretation. We therefore recommend the simultaneous use of Variants I and II Assays, or a reflex test with Variant II Assay depending on the results observed with the Variants I Assay.

The Novaplex™ SARS-CoV-2 Variants II Assay is a major advance in the rapid detection of variants by RT-qPCR. It distinguishes the VOC B.1.351 from the VOC P.1 by amplification of the K417N and K417T mutations respectively when both the N501Y and E484K mutations were found positive with the Variant I Assay. The other two targets of the Variant II Assay are the L452R and W152C mutations. The B.1.617 possess the first mutation, while B.1.427 and B.1.429 possess both mutations. The Variant II Assay does not target the E484Q mutation that would have distinguished B.1.617.1 and B.1.617.3 from B.1.617.2. Nevertheless, one can not preclude a cross-reaction between the E484Q mutation and the detection of the E484K target available in the Variants I Assay. However, this remains mainly hypothetical.

In our workflow, we systematically use the Variants I Assay for any positive sample. Next, we select samples for which no mutation has been detected (to flush out L452R and W152C mutations), as well as samples with an isolated E484K mutation. On the other hand, we do not consider it necessary at this stage to distinguish between B.1.351 and P.1 via the search for mutations in position 417 since the risks of transmissibility, disease severity and reinfection are equivalents.

The superior advantage of mutation detection by RT-qPCR is that there is little viral load limitation on the quality of the result compared to NGS. Indeed, the quality of sequencing becomes poor for Ct above 25. According to the manufacturer's recommendations, we consider positive any mutation amplification result with a Ct less than or equal to 33. On the other hand, while the NGS sequences the genome globally and can clearly identify a VOC or VOI, the mutations targeted by the 2 Variants kits are amplified independently. Therefore, we prefer not to formally name the variants that we detect, but rather to speak of suspected variants.

5. Conclusion

Laboratories with compatible infrastructure are encouraged to use second line mutation screening kits. This makes it possible to monitor in real time the local epidemiology in terms of VOCs. However, care must be taken to ensure that new variants are detected and that VOCs testing kits are regularly updated so as not to miss these newcomers.

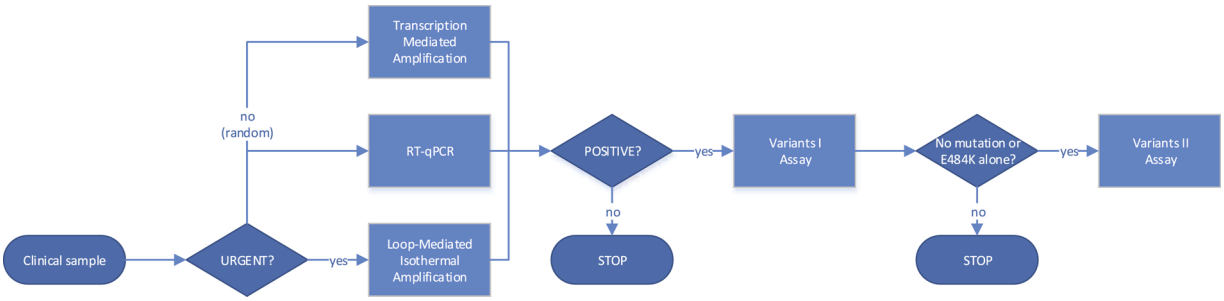

Fig. 1. Workflow of covid-19 analyses.

Fig. 2. Distribution of SARS-CoV-2 mutations detected by the Variants I Assay (A) and recovery of variants B.1.617 with the Variants II Assay (B).

Author statement

Conceived the study: LB and MTH.
Wrote the paper: LB and MTH.
Performed the lab experiments: SP.
Performed the statistical analyses: LB.
Supervised data collection and laboratory work: LB, RC, SP, IB and MTH.
All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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