Clinical Case Reports International

Research Article
Published: 05 Aug, 2024

The Rapid Screening of SARS-CoV-2 Variants can be an Alternative Solution for Low-Income Countries?

Zniber NM, Elbenaissi Y, Aadi Y, Elkochri S, Tagajdid MR, Agoudim S, Elannaz H, Laraqui A, Elmchichi B, Touil N, Ennibi K, Amine IL and Abi R*

Department of Virology, Mohammed V Military Teaching Hospital, Mohammed V University, Morocco

Abstract

This research article assesses *TIB MOLBIOL VirSNiP* molecular screening tests for detecting SARS-CoV-2 variants. The analysis is then compared to high-throughput sequencing results obtained with the Ion Torrent platform. Swab samples from the nasopharynx and oropharynx of COVID-19-positive patients were analyzed in the Virology Laboratory at the Military Teaching Hospital Mohamed V in Rabat. We used RT-PCR and Ion Torrent sequencing for variant detection. The *VirSNiP* assays detected spike mutations N460K, F486P, K444T, NSP12 Y273H, and NSP13 N268S. We assessed concordance between the two-method using Cohen's kappa, sensitivity and specificity. The performance of the Spike N460K and K444T assays was excellent, with a sensitivity and specificity of 100%, thus achieving perfect concordance. Reliability in detecting variants was supported. Performance with the F486P assay was also satisfactory, recording a sensitivity of 89% and specificity of 100% (κ=0.92). However, the sensitivity of both NSP12 Y273H and NSP13 N268S mutation tests was lower at 11% and 30%, respectively, though they remained specific at 100%, with κ values in the moderate range (0.54 and 0.63, respectively). Finally, our results emphasizes that molecular screening is effective in the rapid and precise identification of COVID-19 variants, especially within resource limited institutions.

Impact statement: This research article significantly contributes to the current understanding of molecular screening tests for detecting SARS-CoV-2 variants, especially in resource-limited settings. By comparing the performance of *TIB MOLBIOL VirSNiP* assays against high-throughput Ion Torrent sequencing, our study demonstrates that specific molecular assays, can achieve high sensitivity and specificity providing a reliable and rapid alternative to more expensive and time-consuming methods. This work highlights the utility of molecular screening in offering timely and cost-effective genetic surveillance of SARS-CoV-2 variants, which is crucial for public health interventions and pandemic management. Our findings emphasize the importance of continuous validation and updating of molecular assays to adapt to the evolving genetic landscapes of SARS-CoV-2. The significance of this study lies in its potential to enhance diagnostic capabilities in low-resource settings, thereby supporting global efforts in tracking and controlling the spread of SARS-CoV-2 variants.

Keywords: SARS-CoV-2; NGS sequencing; Variants; Molecular screening; Pandemic management; Real time RT-PCR

OPEN ACCESS

Correspondence:
Zniber Mohamed Nabil, Department of Virology, Mohammed V Military Teaching Hospital, and Mohammed V University, Morocco, Tel: 00212656963820;

Received Date: *18 Jul 2024*
Accepted Date: *01 Aug 2024*
Published Date: *05 Aug 2024*

Citation:
Zniber NM, Elbenaissi Y, Aadi Y, Elkochri S, Tagajdid MR, Agoudim S, et al. The Rapid Screening of SARS-CoV-2 Variants can be an Alternative Solution for Low-Income Countries?. Clin Case Rep Int. 2024; 8: 1697.

Introduction

The ongoing COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has brought significant challenges to public health worldwide. Since its outbreak in December 2019, the virus has infected over 700 million individuals and caused about 7 million deaths worldwide by April 2021 [1,2]. SARS-CoV-2 is an RNA virus with a very high mutation rate, thus showing that several mutations and variants differ significantly in transmissibility, severity, and resistance to interventions in terms of treatment and vaccination. This highly dynamic evolutionary landscape warrants effective, robust, and versatile diagnostic strategies for follow-up and management of the pandemic. This type of genetic surveillance for SARS-CoV-2 is essential for several reasons. It enables the early detection of novel variants, the assessment of their public health impact, and informs strategies for treatment and vaccination [1]. The high level of traditional sequencing methods includes Next-Generation Sequencing (NGS), which is considered the gold standard for identifying and characterizing viral variants due to its high accuracy and comprehensive genomic coverage [3]. These techniques, however, are resource-intensive and take a lot of time, cost, and high

technical expertise.

This is in contrast to the molecular screening tests, which, though accurate, are also fast and less expensive than the *TIB MOLBIOL VirSNiP* assays for detecting variants. These assays also use real-time PCR and target specific mutations associated with known variants to ensure that results are reliable and, most importantly, are availed at a faster turnaround time, which means timely public health interventions [4]. The study thus assesses the performance of the *TIB MOLBIOL VirSNiP* assay in terms of detecting SARS-CoV-2 variants compared to high-throughput sequencing using the Ion Torrent platform. The assays are under evaluation for sensitivity, specificity, and concordance with sequencing results to provide potential implementation as tools for routine surveillance and monitoring of variants.

Materials and Methods

The study was conducted at the Virology Laboratory of the Mohamed V Military Teaching Hospital in Rabat. Based on a real-time RT-PCR assay, nasopharyngeal and oropharyngeal swabs were collected from patients who tested positive for COVID-19. The samples were preserved at -20°C until further processing.

Total nucleic acids were extracted using the *EZ1 Virus Mini Kit v2.0* (QIAGEN, Germany) on the *EZ1 Advanced XL* platform. The extraction process involved mixing 4 µL of diluted carrier RNA with 541 µL of AVE buffer and 20 µL of internal control per sample. 60 µL of this mixture was added to each sample tube, and 400 µL of each sample was loaded into the EZ1 platform. The final elution volume was 60 µL, and the eluates were stored at -20°C until use. The detection of SARS-CoV-2 was performed using the *QuanT Studio 5* instrument (Applied Biosystems) with the *PCL COVID-19 Speedy RT-PCR kit*. This kit targets the N, E, and R genes of SARS-CoV-2.

Ion torrent sequencing

Following nucleic acid extraction using *QIAmp kit* in *EZ1 Advanced XL* (Qiagen), extract were amplified with *PCL COVID-19 Speedy RT-PCR kit* in the *QuantStudi 5 Real-Time PCR Thermocylor*. Using the *Ion AmpliSeq™ Chef Reagent DL8 kit* (Life Technologies, Thermo Fisher Scientific, Waltham, MA REF: A29025) and Ion Chef Reagent (Life Technologies, Thermo Fisher Scientific, Waltham, MA REF: A34018) libraries were prepared. The prepared libraries were then sequenced on the Ion Torrent platform to confirm the presence and type of variants.

TIB MOLBIOL VirSNiP assays

the molecular screening was conducted using *TIB MOLBIOL VirSNiP* assays targeting the following mutations: *Spike N460K, F486P, K444T, NSP12 Y273H*, and *NSP13 N268S*. Each 20 µL reaction mixture for the *VirSNiP* assays contained 5 µL of Master mix, 4 µL of primer/probe mix, 1 µL of RT enzyme, 5 µL of the extracted nucleic acids, and 5 µL of PCR-grade water. The reactions were performed on the *LightCycler 480 II* (Roche) following the manufacturer's instructions. The cycling conditions included an initial denaturation at 95°C for 5 min, followed by 45 cycles of 95°C for 15 sec, 60°C for 30 sec, and a final extension at 72°C for 1 min.

The performance of the *VirSNiP* assays was evaluated by comparing their results with those obtained from Ion Torrent sequencing. Sensitivity, specificity, and concordance (Cohen's kappa coefficient) were calculated for each assay. Concordance was assessed using Cohen's kappa coefficient to measure the agreement between the two methods. Statistical analysis was performed using Excel 2013.

Results

The results section detailed compares the performance of the *TIB MOLBIOL VirSNiP* assays in detecting individual SARS-CoV-2 variants. Comparative performance metrics included sensitivity, specificity, and concordance with Ion Torrent sequencing results. The *TIB MOLBIOL VirSNiP* assay showed 100% sensitivity and 100% specificity for detecting the BQ.1 variant in the case of detection for the Spike N460K mutation. All nine samples of BQ.1 appeared with a melting temperature of 59.5 ± 2°C, while in the case of Cohen's kappa coefficient, it was perfect (κ=1). In the case of the XBB group of 9 samples, they were correctly identified as BA.2.75, which manifested a melting temperature of 57 ± 2°C. The sensitivity of the *VirSNiP* test for detection of the Spike F486P mutation within the XBB VOC was found to be 89%, as it could correctly identify 8 out of 9 XBB samples with a Tm of 65 ± 2°C. On the other hand, the specificity was perfect at 100% since none of the BQ.1 samples were misidentified; however, one XBB sample was called BA.5, resulting in a Cohen's kappa of 0.92. High performance was also seen with the Spike K444T mutation in the *VirSNiP* assay, where 100% sensitivity and specificity were reached for the BQ.1 variant. From the nine BQ.1 samples, eight were identified correctly with an achieved Tm value of 57 ± 2°C, which resulted in a Cohen's kappa of 0.89. All the XBB samples failed to amplify, which means 100% specificity. The *VirSNiP* assay targeted the NSP12 Y273H mutation. Nine BQ.1 samples were found with the Tm of 60 ± 2°C (11%). The specificity was 100%, as no XBB sample was detected, and this low sensitivity resulted in a low Cohen's kappa of 0.54. Detection of the NSP13 N268S mutant in the BQ.1 samples was moderate using the *VirSNiP* assay, with a sensitivity

Table 1: Performances for each VirSNiP assay compared to Ion Torrent sequencing.

Mutation	Variant	True Positives (TP)	False Negatives (FN)	True Negatives (TN)	False Positives (FP)	Sensitivity (%)	Specificity (%)	Cohen's Kappa
Spike N460K	BQ.1	9	0	9	0	100	100	1.00
	XBB	0	9	9	0	0	100	1.00
Spike F486P	XBB	8	1	9	0	89	100	0.92
	BQ.1	0	9	9	0	0	100	0.92
Spike K444T	BQ.1	8	1	9	0	89	100	0.89
	XBB	0	9	9	0	0	100	0.89
NSP12 Y273H	BQ.1	1	8	9	0	11	100	0.54
	XBB	0	9	9	0	0	100	0.54
NSP13 N268S	BQ.1	3	6	9	0	30	100	0.63
	XBB	0	9	9	0	0	100	0.63

of three of nine matched samples (30%) at a Tm threshold of 61 ± 2°C. Consequently, there is 100% specificity if one assumes no XBB cases–the poor sensitivity provided only a Cohen's kappa measure of 0.63. The table below summarizes the performance metrics for each *VirSNiP* assay compared to Ion Torrent sequencing (Table 1).

Discussion

In the context of the COVID-19 pandemic, rapid and accurate detection of SARS-CoV-2. The performance characteristics of molecular screening tests by *TIB MOLBIOL VirSNiP* were evaluated in comparison to high-throughput sequencing (Ion Torrent) to detect specific mutations related to different SARS-CoV-2 variants.

Among the molecular screening tests were the ones tested for the *VirSNiP* assays for spike protein mutations N460K and K444T, and the F486P mutation of spike protein. The other two were NSP12/Y273H and NSP13/N268S, non-structural proteins. There were varying levels of sensitivity and specificity among the tests. Of these, N460K and K444T had 100% sensitivity and 100% specificity, with Cohen's kappa values of 1 and 0.89, respectively. Additionally, the F486P test was also sensitive at 89% and had a kappa value of 0.92 to identify most of the XBB and BQ1 variants accurately.

But some tests mischaracterized the variant because of certain problems:

F486P test misidentifications: In general, the F486P test worked correctly but misidentified various BQ1 samples as BA.5. This is not very surprising since BQ1 was derived from the BA.5 lineage and has, hence, an identical or very similar set of mutations. As a result, the probe recognizing the presence of the F486P mutation in this test cannot distinguish these very similar variants and makes misidentifications.

NSP12/Y273H and NSP13/N268S tests: These performed less well, with sensitivities of 11% and 30%, respectively. It could be an associated mutational event in the regions where the probes will target, thus changing the probe binding sites and hybridization ineffectiveness, leading to false negatives. For the NSP12/Y273H test, among the BQ1 samples, only one was positive. In contrast, for the NSP13/N268S test, three out of the nine BQ1 samples were found to be positive, indicating a probable probe mismatch or suboptimal conditions for the assay.

Scientific literature supports these data. For instance, Alejo-Cancho et al. [5] reported the Mu variant having the K417N mutation, not the Beta variant, by multiplex real-time RT-PCR. They emphasized that workflows must be updated since more defining mutations, like P681H, should be used between those variants.

In support of these findings, a study by Moisan et al. [6] tested the concordance of two in-house RT-PCR for the detection of SARS-CoV-2 variants with NGS data, obtaining an excellent concordance at 99.8% and 99.2% accuracy, respectively, for both assays. A very similar proportion was found by Borillo et al. [7] in which a multiplexed real-time RT-PCR assay was highly concordant to WGS, having 100% concordance for Omicron lineage and other lineages.

Conclusion

The most significant benefit of molecular screening is cost and turnaround time, which offers a platform to be a feasible option for routine surveillance, especially in settings with limited resources. However, this must be combined with the constant validation and updating of molecular assays for the changing viral genomes.

References

1. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. J Med Virol. 2020;92(6):548-51.
2. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021;19(3):141-54.
3. Heather JM, Chain B. The sequence of sequencers: The history of sequencing DNA. Genomics. 2016;107(1):1-8.
4. Mertens J, Coppens J, Loens K, Le Mercier M, Xavier BB, Lammens C, et al. Monitoring the SARS-CoV-2 pandemic: Screening algorithm with single nucleotide polymorphism detection for the rapid identification of established and emerging variants. Clin Microbiol Infect. 2022;28(1):124-9.
5. Alejo-Cancho I, Gual-de-Torrella A, Gallego M, Urrutikoetxea-Gutierrez M, Lejarraga C, López de Goikoetxea MJ. Misidentification of the SARS-CoV-2 Mu variant using commercial mutation screening assays. Arch Virol. 2022;167(4):1141-4.
6. Moisan A, Soares A, De Oliveira F, Alessandri-Gradt E, Lecoquierre F, Fourneaux S, et al. Evaluation of analytical and clinical performance and usefulness in a real-life hospital setting of two in-house real-time RT-PCR assays to track SARS-CoV-2 variants of concern. Viruses. 2023;15(5):1115.
7. Borillo GA, Kagan RM, Marlowe EM. Rapid and accurate identification of SARS-CoV-2 variants using real time PCR assays. Front Cell Infect Microbiol. 2022;12:894613.