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A novel ORF1a-based SARS-CoV-2 RT-PCR assay to resolve inconclusive samples

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ABSTRACT

Background: India bears the second largest burden of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. A multitude of reverse transcription polymerase chain reaction (RT-PCR) detection assays with disparate gene targets, including automated high-throughput platforms, are available. Varying concordance and interpretation of diagnostic results in this setting can result in significant reporting delays, leading to suboptimal disease management. This article reports the development of a novel ORF1a-based SARS-CoV-2 RT-PCR assay – Viroselect – that shows high concordance with conventional assays and the ability to resolve inconclusive results generated during the peak of the epidemic in Mumbai, India.

Methods: A unique target region within SARS-CoV-2 ORF1a — the non-structural protein 3 (nsp3) region — was used to design and develop the assay. This hypervariable region (1923–3956) between SARS-CoV-2, SARS-CoV-1, and Middle East respiratory syndrome coronavirus was utilized to design the primers and probes for the RT-PCR assay. The concordance of this assay with commonly used emergency use authorization (US Food and Drug Administration) manual kits and an automated high-throughput testing platform was evaluated. Further, a retrospective analysis was carried out using Viroselect on samples reported as ‘inconclusive’ between April and October 2020.

Results: In total, 701 samples were tested. Concordance analysis of 477 samples demonstrated high overall agreement of Viroselect with both manual (87.6%) and automated (84.7%) assays. Also, in the retrospective analysis of 224 additional samples reported as ‘inconclusive’, Viroselect was able to resolve 100% (19/19) and 93.7% (192/205) of samples which had inconclusive results on manual and automated high-throughput platforms, respectively.

Conclusion: Viroselect had high concordance with conventional assays, both manual and automated, and has potential to resolve inconclusive samples.

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ARTICLE INFO

Article history:
Received 4 February 2021
Received in revised form 31 March 2021
Accepted 4 April 2021

Keywords:
SARS-CoV 2
RT-PCR
Inconclusive samples
Viroselect
High throughput SARS-CoV-2 testing
Reporting delay

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Introduction

The striking difference between the previous severe acute respiratory syndrome coronavirus (SARS-CoV-1)/Middle East respiratory syndrome coronavirus (MERS-CoV) outbreaks and the ongoing SARS-CoV-2 pandemic is the increased contagious nature of the latter (Yang et al., 2020). This has resulted in over 100 million SARS-CoV-2 infections globally and approximately 2.2 million deaths to date (WHO, 2021). India bears the second largest burden of infected individuals, with 10 million infections and over 150,000 deaths to date (ICMR, 2021). Efficient and rapid diagnosis is critical for optimal management of the spread of coronavirus disease 2019 (COVID-19), as well as clinical management. To ensure maximum coverage (WHO, 2020) and accessibility to testing at economically feasible rates, the Government of India has successfully fostered and promoted the development of indigenous diagnostic kits as well as integrated cutting-edge high-throughput testing facilities (Gupta et al., 2020; Poljak et al., 2020). The availability of a range of diagnostic kits with disparate viral targets has posed a challenge in terms of concordance of results as well as inconclusive results (Bhattacharya et al., 2020; Hur et al., 2020; Pujadas et al., 2020). In addition, implementation of high-throughput platform-based testing, while increasing diagnostic capability, has the potential to generate inconclusive results. As a designated national centre of excellence for COVID-19 diagnostic kit validation as well as a high-throughput testing centre, and having encountered the aforementioned issues, the authors developed an in-house reverse transcription polymerase chain reaction (RT-PCR) assay – ViromeSelect – which was evaluated for concordance with commonly used emergency use authorization (EUA) [US Food and Drug Administration (USFDA)] manual kits and the high-throughput COBAS 6800 testing platform. In addition, the use of ViromeSelect to resolve inconclusive results from both testing modalities was assessed.

Figure 1. (a) Depiction of the hypervariable region between the genomes of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV). Genome alignment was done using align2 blast megablast for SARS CoV and SARS-CoV-2. MERS-CoV-2 and SARS-CoV-2 used align2 blast discontinuous megablast for genome alignment as megablast showed no significant similarity for the alignment. Low alignment scores >40 and 0 describe the hypervariable region between SARS-CoV-2 and SARS-CoV, and SARS-CoV-2 and MERS-CoV. (b) Depiction of primer positions major mutation hotspots of the ORF1ab gene of SARS-CoV-2: (i) the ORF1ab gene is depicted with the nsp regions. The coordinates of ORF1ab genes are shown. (ii) The nsp2 region is highlighted, the binding sites of the forward primer (FP), probe and reverse primer (RP) are shown. (iii) Major single nucleotide variants (SNVs; mutation hotspots) identified in a selected sample size of 673,836: C3037T (96.6%), C3267T (21.4%), C5388A (21.2%), C5986 (21.5%), C6286T (17.9%) and T6954C (21.1%). Samples were collected across all the populated continents. The software used for the analysis was Covid-19 CG.
Materials and methods

VirotecSelect assay development

Primer and probe design
A novel strategy (described in the Results section) was used to design primers to detect SARS-CoV-2. These sequence regions were unique to SARS-CoV-2 but had maximum variation with SARS-CoV and MERS-CoV. More than 200 genomes of SARS-CoV and MERS-CoV were compared with SARS-CoV-2 to identify the hypervariable regions. The SARS-CoV and SARS-CoV-2 genome alignment using NCBI blastn align2 blast, showed highly similar blast sequences (megablast) with an alignment score <40 for the hypervariable region (1923–3956) of SARS-CoV-2. The MERS-CoV and SARS-CoV-2 genome alignment using align2 blast megablast showed no significant similarity. More dissimilar blast sequences (discontiguous megablast) or somewhat similar blast sequences (blastn) used for MERS CoV and SARS-CoV-2 genome alignment did not show any alignment score for the variable region (1923–3956) of SARS-CoV-2 (Figure 1a). The primers were designed using Primer-BLAST NCBI with default parameters. The primers and probes were optimized for an in-house VirotecSelect assay described below. All primers and probes used in this study were synthesized by Macrogen Inc., Seoul, Korea.

SARS-CoV-2 detection

RNA extraction
Oropharyngeal/nasopharyngeal swab samples collected from suspected cases of COVID-19 in viral transport medium (VTM) were used for SARS-CoV-2 detection. Viral RNA extraction from these VTM samples was carried out using a Maverick Magnetic Bead Based Nucleic Acid Extraction Kit (Cat. No.: EC0001B, MyLabs Discovery Solutions Pvt. Ltd., Lonavla, Maharashtra, India) on a Thermo Scientific KingFisher Flex Purification System and a MagRNA-Il Viral RNA Extraction Kit (Cat. No.: G2M030420, Genes2Me Pvt. Ltd, Gurugram, Haryana, India) on an MGISP-100 automated nucleic acid extraction and purification system (MGI Tech Co., Ltd., Yantian, Shenzhen, China), in accordance with the manufacturers’ instructions.

VirotecSelect assay
Biplex RT-PCR assay was conducted for the VirotecSelect assay. The ORF1a (nsP3) gene for the detection of SARS-CoV-2 virus along with human RNase P was used as the internal control from human patient samples. The primer and probe concentrations were as follows: 0.5 μmol/L for primers and 0.5 μmol/L for probes for ORF1a (nsP3); and 0.5 μmol/L for primers and 0.2 μmol/L for probes for human RNaseP. The TaqMan probe was labelled with 6-carboxyfluorescien (6-Fam) 5’ and BHQ1 3’ for ORF1a, and with Cy5 at the 5’ end and BHQ3 at the 3’ end for the RNase P probe. A One Step PrimeScript RT-qPCR kit (Catalogue number: RR600A, Takara Bio, Kyoto, Japan) along with gene specific primers (ORF1a and RNaseP) was used for real-time RT-PCR reaction, in accordance with the manufacturers’ protocol. The one-step RT-PCR conditions were: 52 °C for 5 min (one cycle for reverse transcription), 95 °C for 10 s (initial denaturation), 95 °C for 5 s and 60 °C for 30 s (PCR reaction of 40 cycles).

Assay interpretation for VirotecSelect
The in-house VirotecSelect assay was considered positive if ORF1a (nsP3) and human RNase P (internal control) were detected; negative if ORF1a (nsP3) was not detected but RNase P was detected; and invalid if RNase P was not detected. Interpretation of the STANDARD M nCoV Real-Time Detection Kit (SD Biosensor, Gyeonggi-do, Republic of Korea) and LabGun (LabGenomics Co. Ltd., Gyeonggi-do, Republic of Korea) assays was in accordance with the manufacturers’ instructions.

Interpretation of high-throughput COBAS 6800 SARS-CoV-2 detection assay
In accordance with the manufacturer, four results were possible: positive, negative, invalid and inconclusive. If target 1 (ORF), target 2 (E) and IC (non-infectious RNA in MS2 bacteriophage) were detected, the sample was interpreted as positive; and if both targets were not detected but IC was detected, the sample was interpreted as negative. If the samples were target 1 negative, target 2 positive and IC positive, the sample was interpreted as inconclusive.

Data analysis
Contingency tables were constructed, and positive and negative concordance along with overall agreement between various assays was determined.

Results

Development of novel in-house VirotecSelect assay
Primers were designed using the SARS-CoV-2 reference genome available at NCBI Genbank (Accession No.: NC 045512). The primers and probes were uniquely designed for the VirotecSelect assay from regions which had maximum variation between

Table 1

| Seq ID | Primer name | Primer sequence (5’-3’) | Map location | Strand | Homology with human (QC+ID) | e Value | Amplicon size | Description |
|-------|-------------|------------------------|--------------|--------|-----------------------------|--------|--------------|-------------|
| 1     | ORF1 (nsP3) | TTCTGCTGCTCTCAACCTGA  | 3160–3180    | (-)    | 80%QC, 100% ID             | 1.3    | 174          | Unique to SARS-CoV-2 |
| 2     | ORF1 (nsP3) | ATAGTCTGCAACATTGTTAAGT | 3310–3330    | (+)    | 79%QC, 100% ID             | 2.2    |              |             |
| 3     | ORF1 Probe  | GTTGGTTCAACAAAGCGGAGAGAATC (5’ Tamra-3’ BHQ1) | 3230–3257 | (-)          | No significant similarity | NA     |              |             |
| 4     | Human RNase P | AGATTGGGAGACGGGACCG | Chromosome 10: 90872002–90872020 | (-) | Emery et al. (2004) |
| 5     | Human RNase P | GACGCGGCTCTCGAGCAGCAGC | Chromosome 10: 90872047–90872066 | (+) |             |
| 6     | Human RNase P | TTCTGACCTGAAGGCTGTCGCGC | Chromosome 10: 90872023–90872045 | (-) |             |

QC: Query coverage; ID: percentage identity.
SARS-CoV-2 and MERS-CoV-2. This hypervariable region was identified through NCBI align2 blast by comparing the alignment of more than 200 genomes of SARS-CoV-2 with the same number of SARS-CoV and MERS-CoV genomes (Figure 1a). As shown in Table 1, the sequence targets for designing the primers and probes were present in the ORF1a (nsp3) region of the SARS-CoV-2 genome between nucleotide positions 1923 and 3956. The internal control primer and probe were derived from the human ribonuclease (RNase) P gene (GenBank Accession No.: NM_006413) sequence (Emery et al., 2004). As shown in Figure 1a, the unique design of the primer can aid in differentiating between SARS-CoV and SARS-CoV-2 during mixed infection.

NCBI nBLAST was used to analyse the sequence homology of putative amplicons with >250 SARS-CoV-2 isolates, and 100% nucleotide sequence similarity was observed. Also, primers were designed to ensure that the putative amplicon did not overlap with known major mutation hotspots prevailing globally and in India (Figure 1b). A literature survey and COVID-19 CG software were used for the mutation analysis (covidcg.org) (Chen et al., 2021). The sample size for the mutation analysis for CoVid-19 CG was 673,836. Samples were selected from all continents (Asia, Africa, North America, South America and Australia). Samples were collected between 22 December 2019 and 14 March 2021. No major mutation hotspots were observed in the nsp3 region. However, three mutations of minor frequency – C3177T (1.47% number 9910, forward primer region), T3256C (0.65% number 4372, probe binding region) and C3311T (0.31% number 2064, reverse primer region) – were identified. The position of the mutations in the primers will not affect the binding and detection of the target significantly. The major mutation hotspots for the nsp3 region are shown in Figure 1b.

In addition, care was taken to have minimum homology with the human genome to avoid non-specific amplification (Table 1). Following primer design, an amplicon was generated from infected samples and verified by agarose gel electrophoresis to be of the expected size (Figure S1, see online Supplementary material).

**Concordance analysis**

**Viroselect with SD Biosensor assay**

To assess the concordance of Viroselect with established and approved kits used for diagnosis, the overall agreement of Viroselect was compared with an EUA (USFDA)-approved kit (SD Biosensor). RNA extraction from samples diagnosed as positive (n = 126) and negative (n = 107) for SARS-CoV-2 infection by SD Biosensor was followed by RT-PCR using Viroselect. Results revealed high concordance of Viroselect with SD Biosensor, as summarized in Table 2. As shown in Figure 2, a total of 286 samples (including 19 inconclusive samples) were tested, of which 34 were invalid (not included in the analysis). Concordance of 92.9% among positive samples and 81.3% among negative samples was observed. Overall agreement of 87.6% [95% confidence interval (CI) 74.91–93.62%] was observed (Table 2). Interestingly, a higher rate of discordance was observed in negative samples (20/107; 18.7%) compared with positive samples (9/126; 7.1%).

**Table 2** Concordance of SD Biosensor-positive and -negative samples with Viroselect.

| Viroselect results | SD Biosensor | Concordance among positives | Concordance among negatives | Overall agreement (%) (95% CI) |
|-------------------|--------------|-----------------------------|-----------------------------|-----------------------------|
| Positive          | 117          | 137                         | 92.85%                      | 81.31%                      |
| Negative          | 9            | 96                          | 87.55% (74.91–93.62)        |
| Total             | 126          | 233                         |                             |                             |

CI, confidence interval.

Notably, of the 19 inconclusive results using SD Biosensor, seven (37%) were positive on Viroselect and 12 were negative. Of the seven positive results, six were positive when tested with another EUA (USFDA)-approved RT-PCR kit (LabGun COVID-19 RT-PCR Kit; LabGenomics). Further, clinical data obtained from the ICMR specimen referral form revealed that six of seven individuals that tested positive on Viroselect were symptomatic (Figure 2). Thus, Viroselect was able to resolve 100% of samples that had inconclusive results on SD Biosensor testing.

**Viroselect assay with COBAS 6800 high-throughput platform**

As the authors’ centre transitioned to a high-throughput testing facility, the opportunity arose to establish the concordance of Viroselect with the SARS-CoV-2 detection assay on the COBAS 6800 high-throughput platform (COBAS). Known COBAS positive (n = 102) and negative (n = 108) samples were tested by Viroselect. Seven samples were found to be invalid and were not analysed further. The results revealed high concordance of Viroselect with COBAS, as summarized in Table 3. Concordance of 89% for positive samples and 84.5% for negative samples was observed, with overall agreement of 84.7% (95% CI 76.00–90.85%) (Table 3). Interestingly, a higher rate of discordance was observed in negative samples (16/103; 15.5%) compared with positive samples (11/100; 11%).

As shown in Figure 3, a total of 205 inconclusive samples were also tested, of which 13 were invalid (not included in the analysis). Of these, 175 (91.2%) tested negative and 17 (8.8%) tested positive. This indicates that 192 of 205 (93.7%) of the samples that were inconclusive on COBAS were resolved using Viroselect.

**Discussion**

This study demonstrated the use of a unique, ORF1a-based SARS-CoV-2 detection assay to resolve inconclusive results obtained from a conventional manual assay and a high-throughput testing platform.

Real-time PCR has been established as the gold standard for detecting SARS-CoV-2 infection. Whole-genome sequencing of SARS-CoV-2 in very large numbers and availability of free databases (including NCBI and GISAID) to access the genomes...
Table 3
Concordance of COBAS-positive and -negative samples with Viroselect.

| Viroselect results | COBAS results | Concordance among positives | Concordance among negatives | Overall agreement (%) (95% CI) |
|-------------------|---------------|-----------------------------|-----------------------------|-------------------------------|
| Positive          | Positive      | 105                         | 89%                         | 84.47% (76.00–90.85%)         |
|                   | Negative      | 98                          |                             |                               |
| Total             |               | 203                         |                             |                               |

CI, confidence interval.

Figure 3. Flowchart showing Viroselect results for the selected sample panel tested against the COBAS 6800 platform.

have attributed to the development of a multitude of RT-PCR kits which can often provide conflicting and inconclusive results (Fang et al., 2020). Thus, a testing strategy that would resolve these issues would greatly enhance the efficiency of diagnosis, which, in turn, would significantly impact the transmission dynamics and allow efficient epidemiological surveillance. In addition, the rapidly accumulating mutations in the SARS-CoV-2 genome pose a challenge for accurate diagnosis of the infection due to possible mispriming in assays that rely on the detection of viral sequences in these regions (Rambaut et al., 2020). Based on hypervariable sequence analysis as well as mutation hotspot enumeration, the authors designed unique primers capable of differentiating infection with SARS-CoV-2, SARS-CoV and MERS-CoV. The hyperviability was observed in the regions (1923–3956) of ORF1a, and the primer/probe set spanned the nsp3 region. This design strategy was implemented to develop an in-house RT-PCR-based assay for detection of SARS-CoV-2.

The unique primer and probe design were subjected to comparative analysis with: (1) World Health Organization (WHO)-recommended and EUA (USFDA)-approved SD Biosensor kits; and (2) the high-throughput, automated SARS-CoV-2 detection assay on the COBAS 6800 platform. Overall, high concordance was observed for Viroselect with both of these detection modalities. Viroselect was able to resolve 100% of the inconclusive results obtained using SD Biosensor. Notably, seven of 19 (36.8%) of these samples were found to be positive on Viroselect, and six of seven were found to be positive using another EUA (USFDA)-approved kit, LabGun. Additionally, when corroborated clinically, six of seven inconclusive samples that tested positive on Viroselect were documented to have been obtained from symptomatic individuals.

Precise and early diagnosis and treatment is crucial in COVID-19 (Rong et al., 2020). High-throughput diagnostic platforms, such as COBAS 6800, are critical to address diagnostic demand during fast-moving epidemics such as COVID-19, and have a direct impact on curtailing transmission as well as clinical management. Considering the similarity in geography, disease prevalence, population dynamics and healthcare spending of all the countries in South Asia, the Ministry of Health and Family Welfare of the Government of India adopted commendable measures to tackle the COVID-19 pandemic. A major measure was increased accessibility to high-throughput testing facilities. The authors’ laboratory was one of these centres, and close to 100,000 samples from Mumbai and surrounding areas have been tested to date. When dealing with such high throughput, a major challenge encountered was the rate of inconclusive results. Although the rate was relatively low (3%), the absolute number translated into approximately 3000 individuals (for every 100,000 individuals). Considering the rapid transmission dynamics of SARS-CoV-2, even a delay of 1 day in correctly diagnosing and reporting a false-negative or inconclusive sample as positive would have a significant impact on disease transmission in a highly populated setting such as India (Kretzschmar et al., 2020; Larremore et al., 2021; Rong et al., 2020). When Viroselect was used to test a subset of inconclusive samples obtained from the COBAS 6800 platform, it was able to resolve approximately 94% of these samples. Most importantly, approximately 9% of these inconclusive results were found to be positive on Viroselect. This assay would thus enable optimal intervention in managing these cases, especially if they are assumed to be potential transmitters. While this study demonstrates the value of a unique primer-based assay to resolve inconclusive samples, it is acknowledged that this approach needs to be validated with a gold standard and large sample size to confirm its efficacy.

Competing interests

None declared.

Funding sources

This work was supported by Indian Council of Medical Research-National Institute for Research in Reproductive Health. The funding body had no role in the design of the study, collection, analysis and interpretation of data or in writing the manuscript.

Ethical approval

This study was approved by Institutional Ethics Committee at ICMR-NIRRH (Project No. 399-2020)

Authors’ contributions

DJ, SB1 and VP conceptualized the study. KJ, AYS, BC, SK, NK, SB2, SB3, PD, KK and HP performed the experiments. KJ, AYS and BC conducted primary data analysis and prepared the figures. JS, SA, KJ, BC and KM collected the clinical data. DJ, SB1 and VP supervised the study. VP and DJ wrote and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

Acknowledgments

The authors wish to thank the Director General, Indian Council of Medical Research, Ministry for Health and Family Welfare,
Government of India for encouragement and support to answer important research questions relevant to COVID-19 pathogenesis.

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.ijid.2021.04.006.

References

Bhattacharya S, Vidyadharan A, Joy V. Inconclusive SARS-COV-2 reverse transcription polymerase chain reaction test reports: interpretation, clinical and infection control implications. J Acad Clin Microbiol 2020;22:59–61.

Chen AT, Altschuler K, Zhan SH, Chan YA, Deverman BE. COVID-19 CG enables SARS-CoV-2 mutation and lineage tracking by locations and dates of interest. Elife 2021;10:e53409.

Emery SL, Erdman DD, Bowen MD, Newton BR, Winchell JM, Meyer RE, et al. Real-time reverse transcription-polymerase chain reaction assay for SARS-associated coronavirus. Emerg Infect Dis J 2004;10:311.

Fang FC, Naccache SN, Greninger AL. The laboratory diagnosis of coronavirus disease 2019 — frequently asked question. Clin Infect Dis 2020;71:2996–3001.

Gupta N, Bhatnagar T, Rade K, Murhekai M, Gangakhedkar RR, Nagar A. Strategic planning to augment the testing capacity for COVID-19 in India. Indian J Med Res 2020;151:210–5.

Hurl K-H, Park K, Lim Y, Jeong YS, Sung H, Kim M-N. Evaluation of four commercial kits for SARS-CoV-2 real-time reverse-transcription polymerase chain reaction approved by emergency-use-authorization in Korea. Front Med 2020;7:521.

ICMR. COVID-19 testing status. Delhi: Indian Council of Medical Research; 2021 Available at: https://www.icmr.gov.in/pdf/covid/update/ICMR_testing_update_29Jan2021.pdf. [Accessed 29 January 2021].

Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Roon M, van de Wijgert JHJM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. Lancet Public Health 2020;5: e452–9.

Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, Hay JA, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. Sci Adv 2021;7: eabd5393.

Poljak M, Korva M, Knop Galper N, Fugls Komiš K, Sagadin M, Urtš T, et al. Clinical evaluation of the cobas SARS-CoV-2 test and a diagnostic platform switch during 48 hours in the midst of the COVID-19 pandemic. J Clin Microbiol 2020;58: e00599–20.

Pujadas E, Bnh H, Hernandez MM, Waluszko A, Sidorenko T, Flores V, et al. Comparison of SARS-CoV-2 detection from nasopharyngeal swab samples by the Roche cobas 6800 SARS-CoV-2 test and a laboratory-developed real-time RT-PCR test. J Med Virol 2020;92:1695–8.

Rambaut A, Loman N, Pybus O, Barclay W, Barrett J, Carabelli A, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. ARTIC Network, COVID-19 Genomics Consortium UK (CoG-UK); 2020 Available at: https://virological.org/p/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563. [Accessed 14 April 2021].

Rong X, Yang L, Chu H, Fan M. Effect of delay in diagnosis on transmission of COVID-19. Math Biosci Eng 2020;17:2725–40.

WHO. Feature story: how India scaled up its laboratory testing capacity for COVID-19. Geneva: World Health Organization; 2020 Available at: https://www.who.int/india/news/feature-stories/detail/how-india-scaled-up-its-laboratory-testing-capacity-for-covid19. [Accessed 14 April 2021].

WHO. Coronavirus disease (COVID-19) dashboard. Geneva: World Health Organization; 2021 Available at: https://covid19.who.int/. [Accessed 28 January 2021].

Yang Y, Peng F, Wang R, Yange M, Guan K, Jiang T, et al. The deadly coronaviruses: the 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. J Autoimmun 2020;109:102434/detail/how-india-scaled-up-its-laboratory-testing-capacity-for-covid19.

WHO. 2021WHO. Coronavirus disease (COVID-19) dashboard Available at: https://covid19.who.int/. [Accessed 28 January 2021].

Yang et al., 2020Yang. F.Peng, R.Wang, M.Yange, K.Guan. T.Jiang. The deadly coronaviruses: the 2003 SARS pandemic and the 2020 novel coronavirus epidemic in ChinaJ Autoimmun109:102434.