



SHORT COMMUNICATION

Use of saliva and RT-PCR screening for SARS-CoV-2 variants of concern: Surveillance and monitoring

Rodrigo Melim Zerbinati¹  | Michelle Palmieri² | Gabriela Schwab¹ |
Alvina Clara Felix¹ | Herculano Martinho³ | Simone Giannecchini⁴ |
Kelvin Kai-Wang To⁵ | Jose Angelo Lauletta Lindoso^{6,7,8} | Camila Malta Romano¹ |
Paulo Henrique Braz-Silva^{1,2} 

¹Laboratory of Virology (LIM-52-HCFMUSP), Institute of Tropical Medicine of São Paulo, University of São Paulo School of Medicine, São Paulo, Brazil

²Department of Stomatology, University of São Paulo School of Dentistry, São Paulo, Brazil

³Federal University of ABC, Santo André, Brazil

⁴Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

⁵State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology, Carol Yu Centre for Infection, Li Ka Shing Faculty of Medicine of the University of Hong Kong, Special Administrative Region, Hong Kong, China

⁶Emílio Ribas Institute of Infectious Diseases, São Paulo, Brazil

⁷Laboratory of Protozoology (LIM-49-HC-FMUSP), Institute of Tropical Medicine of São Paulo, University of São Paulo School of Medicine, São Paulo, Brazil

⁸Department of Infectious Diseases, University of São Paulo School of Medicine, São Paulo, Brazil

Correspondence

Paulo Henrique Braz-Silva, Laboratory of Protozoology (LIM-49-HC-FMUSP) of the Institute of Tropical Medicine of São Paulo, School of Medicine, University of São Paulo-Av. Dr. Enéas Carvalho de Aguiar, 470-05403-000, São Paulo, Brazil.
Email: pbraz@usp.br

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Abstract

Genomic surveillance has been applied since the beginning of the COVID-19 pandemic to track the spread of the virus, leading to the characterization of multiple SARS-CoV-2 variants, including variants of concern (VOC). Although sequencing is the standard method, a rapid molecular test for screening and surveillance of VOC is considered for detection. Furthermore, using alternative saliva as specimen collection facilitates the implementation of a less invasive, self-collected sample. In this study, we applied a combinatory strategy of saliva collection and reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 VOC detection. Saliva samples from patients attending a tertiary hospital with suspected COVID-19 were collected and SARS-CoV-2 RNA was detected using SARS-CoV-2 RT-qPCR reagent kit (PerkinElmer). Positive saliva samples were screened for SARS-CoV-2 VOC with previously described RT-PCR for Alpha, Beta, and Gamma variants. Saliva samples were positive in 171 (53%) of 324 tested. A total of 108 (74%) from positive samples were also positive for VOC by RT-PCR screening. Those samples were found between January and August 2021. This approach allowed us to successfully use an alternative and complementary tool to genomic surveillance to monitor the circulation of SARS-CoV-2 VOC in the studied population.

KEYWORDS

saliva, SARS-CoV-2, surveillance, variants of concern

1 | INTRODUCTION

As the COVID-19 pandemic continues and new SARS-CoV-2 variants emerge, it is necessary to develop strategies to track them, particularly when they enter new territories.¹ Several genomes have been shared since the beginning of the pandemic. The analysis of these sequences has allowed multiple variants to be categorized,² including the variants of concern (VOC): Alpha, Beta, Gamma, Delta, and more recently, Omicron (<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>). In Brazil, Alpha circulated very briefly but in late 2020 Gamma emerged, in the Amazon city of Manaus and has spread quickly over the country, thus becoming the dominant lineage for months.^{2,3}

The use of complementary tools for genomic surveillance based on next-generation sequencing such as reverse transcription polymerase chain reaction (RT-PCR) is an important, accessible, and economical strategy to monitor VOCs. Among these strategies are those using gene target failure, which is based on deletion in the region of the assay. For instance, the commercial assay TaqPath (Thermo Fisher Scientific) identifies Alpha and more recently Omicron, despite being not designed for this specific purpose. There are also in-house assays for the identification of circulating VOC based on similar strategies.^{1,4}

Although nasopharyngeal swab (NPS) is the standard method for laboratory COVID-19 diagnosis, saliva analysis has been pointed out as an alternative technique for the detection of SARS-CoV-2 RNA. Moreover, saliva has important advantages: less invasiveness, possibility of self-collection, and easy implementation of serial collections.^{5,6} The use of saliva as an alternative sample for SARS-CoV-2 detection by using RT-PCR has been explored since the beginning of the pandemic.⁶ The sensitivity of saliva compared to NPS range from 78% to 100%, showing that saliva can be used as a reliable sample for SARS-CoV-2 detection, especially on population-based tests.⁷ However higher cycle threshold (C_t) values have been noticed in saliva.^{8,9}

Here, we assessed the use of a strategy combining saliva collection, SARS-CoV-2 diagnostic, and RT-PCR specific for VOC in the follow-up of a population attending a tertiary hospital.

2 | MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Emilio Ribas Institute of Infectious Diseases, São Paulo, Brazil, according to protocol CAAE 35589320.6.0000.0061 and carried out in accordance with the Helsinki Declaration. Patients with clinical manifestations of COVID-19 who received first-aid care (outpatient), hospitalization care (inpatient), or intensive care unit (ICU) at ERIID were included in the study. They were instructed to chew a Salivette[®] cotton pad (Sarstedt AD & Co). for 1 min for saliva collection. For patients under mechanical ventilation, a dentist, by using a tweezer, maintain the cotton pad in the oral cavity of the patient also for 1 min. Salivette[®] were centrifuged at 1000g for 2 min to retrieve the saliva.

Extraction of total nucleic acid was performed from 200 μ l of the saliva by using the PureLink[™] viral RNA/DNA mini kit (Invitrogen), whereas RNA was detected through the SARS-CoV-2 RT-qPCR reagent kit (PerkinElmer). The samples were considered positive when one or more targets (N gene and ORF1ab gene) had a C_t value ≤ 42 , as stated by the manufacturer.

The positive saliva samples were screened for VOC by using in-house RT-PCR previously described,⁴ which uses a pair of primers and two probes to differentiate wild variants (designated here as non-VOC) from the three known VOC at that time (Alpha, Beta, and Gamma) without, however, discriminating them. Results were interpreted according to reference procedures.⁴ The PKamp[™] variant detect[™] SARS-CoV-2 RT-PCR assay (PerkinElmer) was used in VOC positive samples to classify them as Alpha, Beta, or Gamma.

3 | RESULTS

A total of 324 patients attending the ERIID between January 13 and December 8, 2021, were included in the study and had their saliva collected. Of these patients, 148 saliva samples were from the first-aid room (outpatient), 88 from the hospital ward (inpatient), and 88 from the intensive care unit (ICU). Male patients accounted for 56.2% ($n = 182$) of the participants and female for 43.8% ($n = 142$). The mean age was 49.7 ± 14.7 years old (ranging from 19 to 88 years). The mean time between symptoms and saliva collection was 8.9 ± 6.0 days (interval of 0–43 days). A total of 171 (53%) saliva samples were positive among the 324 tested using RT-PCR for SARS-CoV-2, with C_t values ranging from 16.7 to 38.6 (mean of 30.4 ± 5.3) for the N gene and from 16.0 to 39.1 (29.7 ± 5.6) for ORF1ab gene. Positive samples were found in 57.3% ($n = 98$) of the male patients and in 42.7% ($n = 73$) of the female patients. The mean time between the onset of the symptoms and saliva collection was 9.6 ± 4.6 days, with an interval of 0–24 days. Negative samples were 8.1 ± 7.2 days (interval 0–43 days) (Figure 1A).

Positive saliva samples ($n = 171$) were submitted to the RT-PCR screening for VOC. The amplification curve was observed in 84.8% ($n = 145$) in one of the two targets (i.e., non-VOC or VOC). VOC (Alpha, Beta, or Gamma) was detected in 108 (74%) samples, and 37 (26%) were typed as non-VOC. Positive samples in which C_t value was < 35 ($n = 91$) for VOC screening were tested for characterization with PKamp[™] variant detect[™] SARS-CoV-2 RT-PCR assay (PerkinElmer). Of these, a total of 85 (93%) samples were Gamma and 6 (7%) were inconclusive or negative. Detection of VOC varied throughout the time with predominance between March and July (76.9%) and non-VOC were mostly found in January, February, and August (Figure 1B).

4 | DISCUSSION

In this study, it was possible to identify the circulation of SARS-CoV-2 VOC by using RT-PCR screening and noninvasive saliva sampling from symptomatic patients attending a tertiary hospital. Out of 171

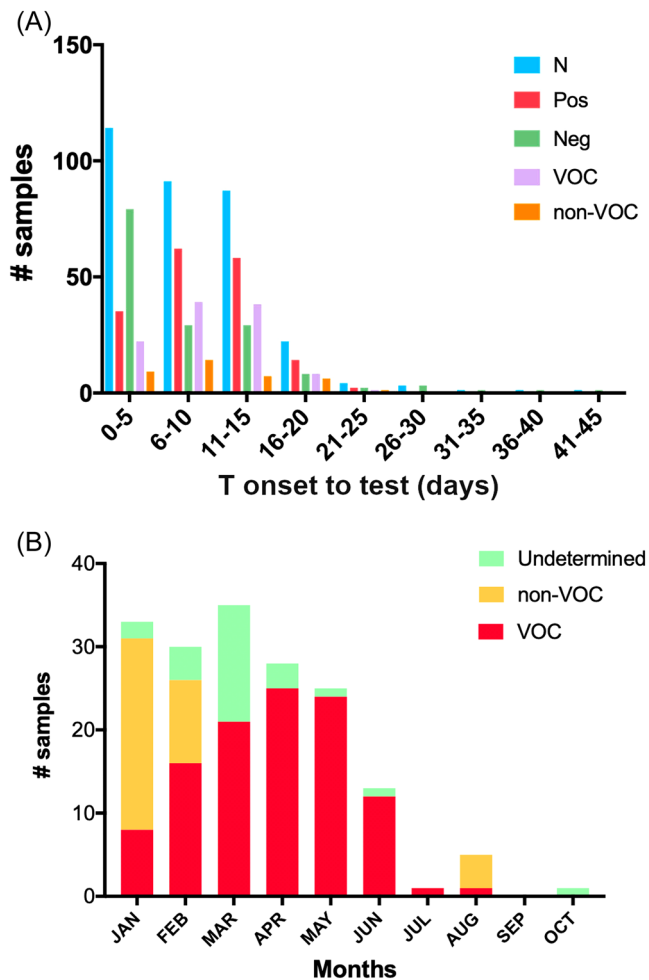


FIGURE 1 Saliva samples distribution in (A) Interval of days between the onset of symptoms and saliva collection (test); (B) Detection of variants in positive saliva samples throughout the time since the onset of the symptoms (months/2021).

N (total of samples), Pos (positive samples in SARS-CoV-2 RT-PCR assay), Neg (negative samples in SARS-CoV-2 RT-PCR assay), VOC (positive for SARS-CoV-2 variants of concern RT-PCR screening), and non-VOC (positive for wild variants SARS-CoV-2 RT-PCR screening). RT-PCR, reverse transcription polymerase chain reaction

positive saliva samples, SARS-CoV-2 VOC (Alpha, Beta, or Gamma) was detected in 145 patients by using a strategy combining RT-PCR screening assays. Therefore, it was confirmed the early detection in January of the VOC Gamma and its dominance in the period between March and July 2021.

The genomic analysis is extremely important for follow up adequately an epidemic such as the COVID-19 and the rapid spread of SARS-CoV-2 variants.² Although genomic surveillance is the most used and standard method for variant detection, it is dependent on resources and expertise. Therefore, the use of complementary tools, for example, RT-PCR screening, for the detection of VOC is important to monitor their transmission and circulation, as similarly described in Vogels et al.¹ This method allowed not only assessment of the presence/absence of VOC in approximately 85% of the

positive samples but also monitored the emergence and dominance of Gamma between March and July 2021, which is consistent with studies using sequencing data.¹⁰ A recent study showed that saliva is also highly sensitive for detecting Beta variants.¹¹

Saliva sampling has been increasingly used as an alternative to the standard nasopharyngeal swab for the detection of SARS-CoV-2. It showed excellent sensitivity for detecting SARS-CoV-2 VOC when RT-PCR screening was used in this study. The method is an easy-to-use noninvasive technique with proven sensitivity and allows self-collection.⁵ Although the collection time in relation to the onset of symptoms can impact the viral RNA detection,¹² the results remain unaffected. Hence, saliva can be considered the sample of choice for studies aimed at monitoring the circulation of SARS-CoV-2 lineages.

The combination of a method of noninvasive (self-) collection (with the possibility of serial collection) and RT-PCR screening for SARS-CoV-2 VOC, or even variants of interest, would be of great importance for assisting in the genomic surveillance. Considering its low cost and less complexity, saliva sampling can be implemented in regions with low resources or competencies available.

Despite the monitoring of the Gamma circulation, it was observed a decrease in cases from September in the city of São Paulo (outbreak.info), which impact the number of patients attendance and saliva collection in the site of the study. Considering this limitation, it was not possible to assess the introduction of Delta or possible cases of Omicron in the study population. The introduction and replacement of Gamma in the Brazilian population by Delta occurred in the second half of 2021.¹³

Our results have demonstrated the successful use of RT-PCR in saliva samples for the evaluation of VOC circulation in patients with COVID-19. This method allows the use on a complimentary basis of genomic surveillance, to monitor the epidemiological scenario during the pandemic in specific regions or services.

AUTHOR CONTRIBUTIONS

Conception and design: Rodrigo Melim Zerbinati and Paulo Henrique Braz-Silva. *Investigations:* Rodrigo Melim Zerbinati, Michelle Palmieri, Gabriela Schwab, Alvina Clara Felix. *Data Analysis:* Rodrigo Melim Zerbinati, Michelle Palmieri, Herculano Martinho, Camila Malta Romano, Paulo Henrique Braz-Silva. *Writing—Original:* Rodrigo Melim Zerbinati. *Draft preparation:* Rodrigo Melim Zerbinati, Michelle Palmieri, Paulo Henrique Braz-Silva. *Revision:* Herculano Martinho, Simone Giannecchini, Jose Angelo Lauletta Lindoso, Camila Malta Romano, Paulo Henrique Braz-Silva. *Supervision:* Paulo Henrique Braz-Silva. All authors reviewed and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Rodrigo Melim Zerbinati  <http://orcid.org/0000-0001-9936-9139>

Paulo Henrique Braz-Silva  <http://orcid.org/0000-0002-1842-9521>

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