Molecular identification of SARS-CoV-2 on environmental surfaces in healthcare facilities of a public university in Brazil

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ABSTRACT
On March 11, 2020, the World Health Organization (WHO) decreed the pandemic of COVID-19, caused by the SARS-CoV-2 virus, responsible for more than 4.5 million deaths to date. This new reality demanded responses from the authorities and the population in order to mitigate the spread of the virus and avoid the collapse of the health system, as well as health surveillance studies, which enabled a better understanding of the mechanisms of virus transmission and made it possible to identify risk zones within cities or public environments. This study aims to identify the presence of SARS-CoV-2 within the Universidade Estadual do Oeste do Paraná, which provides health services to the local population, as well as to perform an internal control at the university's Molecular Biochemistry Laboratory (LaBioqMol), where RT-PCR tests are performed weekly. Twenty-one samples were collected from areas frequently touched by people, and the presence of viral RNA and human genetic material was identified by RT-PCR. In none of the samples was the presence of the virus detected. However, in 8 (38.1%) of the samples the RNaseP gene amplification was verified, indicating the presence of human cells. This study assists in quality control and assurance at LaBioqMol and strengthens the view that environmental contamination by SARS-CoV-2 is probably less frequent than was previously suggested at the beginning of the pandemic.

Keywords: covid-19, rt-pcr, monitoring, transmission, contamination.

1 INTRODUCCIÓN
El virus SARS-CoV-2, coronavirus 2 o COVID-19, es un virus de ARN monocatenario positivo, que tiene un tamaño de 50 a 200 nm y cuya estructura tiene cuatro proteínas estructurales, que pueden ser de membrana (M), de envoltura (E), de espiga (S) y de nucleocápside (N) (Wu et al., 2020). Originado a finales de 2019 en Wuhan, China, el coronavirus 2 se extendió rápidamente por todo el mundo, adquiriendo el estatus de pandemia en marzo de 2020 (Organización Mundial de la Salud, 2020) y, a principios de septiembre de 2021, era responsable de más de 4,5 millones de muertes en todo el mundo (Organización Mundial de la Salud, 2021), unas 580.000 de ellas en Brasil (Consejo Nacional de Secretarios de Salud, 2021).
Basándose en estudios como el de Kampf et al. (2020) y Van Doremalen et al. (2020), que demostró que el coronavirus 2 puede permanecer viable hasta días en diversas superficies inanimadas, así como las pruebas en las que se verificó la presencia del virus en instalaciones sanitarias y en el medio ambiente, se han intensificado las preguntas que implican la posibilidad de transmisión indirecta, lo que hace suponer que este tipo de transmisión puede contribuir a explicar la alta infectividad del COVID-19, aunque no con la misma magnitud que el contacto de persona a persona, especialmente en entornos abiertos (Center for Disease Control and Prevention Division of Viral Diseases, 2021; Kanamori et al., 2020; véase también Liu et al., 2020).

Dado el elevado número de muertes en todo el mundo y las características de transmisión del virus, resulta esencial adoptar medidas que contribuyan a la detección de nuevos casos, el seguimiento de las tendencias y la evaluación del riesgo es de suma importancia no sólo para comprender mejor el SRAS-CoV-2, sino también para poder combatirlo con la mayor eficacia posible (véase también Mohammed, 2020; Waldman y Rosa, 1998).

Así, este trabajo tiene como objetivo identificar la presencia del virus SARS-CoV-2 en el ambiente de la Universidade Estadual do Oeste do Paraná, así como evaluar las Buenas Prácticas de Laboratorio del Laboratorio de Bioquímica Molecular de la Unioeste, utilizando la técnica de RT-PCR.

2 MATERIALES Y MÉTODOS

La recogida de las 21 muestras se produjo en dos días diferentes, donde 7 de ellas se tomaron en el Laboratorio de Bioquímica Molecular de Unioeste (LaBioqMol), 2 en el depósito de residuos infecciosos, 3 en el sector de fisioterapia, 1 en el sector de odontología y 1 en la Facultad de Farmacia de la universidad, otras 7 muestras se recogieron dentro del ambulatorio para el diagnóstico del Sars-CoV-2 universitario. Algunos de los puntos de recogida se ilustran en las figuras 1 y 2.
Figure 1. photographic image of collection points. (A) RNA extraction room (CQB-NB2) of the Molecular Biochemistry Laboratory; (B) StepOne real-time thermal cycler computer; (c) Reception desk of the physical therapy and dentistry clinics; (C) Scales for body weight measurement of the school pharmacy; (D) Stretcher for the physical therapy and dentistry clinics; (E) Reception chair of the physical therapy and dentistry clinics.

Figure 2 - Representative image of 4 collection points. (A) Collection room of the University Outpatient Clinic; (B) Chair occupied by patients at the time of collection; (C) Light switch at the reception desk of the University Outpatient Clinic; (D) Door handle of the access door to the sample collection room of the Outpatient Clinic.
For this, rayon swabs soaked in sterile 0.9% saline solution or sterile VTM media (Laborclin) were used and rubbed over the area of interest, performing rotatory movements so that the entire surface of the tip of the swab was used. After this step, each swab had the excess of its stem cut off and was dipped in its respective screw-top bottle containing about 3 mL of the solution. The samples were stored at 4 to 8°C for no more than 48 hours until they were submitted to viral RNA extraction.

The extraction was performed with an extraction kit produced by Qiagen, the QIAMP® Viral RNA mini kit. The qRT-PCR was performed as described by the American CDC (Center for Disease Control and Prevention). The assays were performed using the StepOne equipment (Applied Biosystems®), probes and primers (TaqMan-FAM) were purchased from IDT® (Integrated DNA Technologies®), and the QuantiTect Probe qRT-PCR mixer (Qiagen®). The amplified targets were the genes for the SARS-CoV-2 nucleocapsid proteins, N1 and N2, simultaneously with amplification of the human RNaseP gene, RP.

The StepOne equipment provides quantitative results, and for the environmental sample to be considered detectable, it should present a Ct (cycle threshold) less than or equal to 36 for the N1 and N2 genes, and amplification for the human RNaseP (RP) gene with a Ct less than or equal to 40. The analysis of the results obtained was performed considering the baseline set by default in the device and the threshold set at the beginning of the log phase of the amplification curve of the control samples, above any background noise. Control patient samples containing pre-defined amounts of SARS-CoV-2 RNA were used as control for the reaction.

3 RESULTS AND DISCUSSION

As represented in Table 1, all samples collected were non-detectable for SARS-CoV-2, since 0 of 21 (0.0%) samples had a Ct of up to 36 for the N1 and N2 genes. Of these samples, those taken from the laminar flow, the extraction table, the mouse and the sample transfer window of LaBioqMol, the door handle of the physical therapy sector, the reception counter of the Dentistry sector, and the scales of the School Pharmacy showed amplification of the human RNaseP gene, representing 7 of the 14 (50.0%) samples present in the first plate. There was amplification of this gene in only 1 of the 7 samples (14.3%) on the second plate (patient chair). The amplification curves can be seen in figures 4 and 5.
Figure 4: Amplification curve of the 14 samples present in the first experimental plate, plus the positive and negative controls. The threshold (0.02) is shown at the beginning of the exponential amplification phase of the control samples.

![Amplification Plot](image1)

<table>
<thead>
<tr>
<th>Local</th>
<th>Amostras</th>
<th>Positivo para SARS-CoV-2</th>
<th>Amplificação do gene RNaseP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaBioqMol</td>
<td>7</td>
<td>0 (0,00%)</td>
<td>4 (57.1%)</td>
</tr>
<tr>
<td>Depósito de lixo infectante</td>
<td>2</td>
<td>0 (0,00%)</td>
<td>0 (0,00%)</td>
</tr>
<tr>
<td>Setor de fisioterapia</td>
<td>3</td>
<td>0 (0,00%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Setor de odontologia</td>
<td>1</td>
<td>0 (0,00%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Farmácia Escola</td>
<td>1</td>
<td>0 (0,00%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Ambulatório universitário</td>
<td>7</td>
<td>0 (0,00%)</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>0 (0,00%)</td>
<td>8 (38.1%)</td>
</tr>
</tbody>
</table>

Figure 5: Amplification curve of the 7 samples present in the second experimental plate. The threshold (0.015) is automatically indicated by the analysis software at the beginning of the exponential amplification phase of the control samples.

![Amplification Plot](image2)
The results presented in this manuscript are in agreement with those shown by Goldman (2020), who showed that contamination of the environment and consequently indirect transmission of SARS-CoV-2 occurs significantly less than had been theorized, when considering the everyday conditions of healthcare facilities. This is because in studies such as that of Kampf et al. (2020) and Van Doremalen et al. (2020), biological samples were inoculated onto a small surface containing high Sars-CoV-2 load, which is hardly found in real-life conditions, as pointed out by Goldman (2020). It can be suggested that viral transmission occurs preponderantly by the contact relationship between people and less frequently from people to objects.

In a review conducted by Kanamori et al. (2020), the results found were conflicting, pointing to a significant rate of surface contamination in hospital settings, especially in the wards of Covid-19 infected patients. However, detection of the presence of viral genetic material on surfaces does not confirm the viability of the virus, nor the possibility of infection by these means. In another recent review, involving 37 studies, Gonçalves et al. (2021) describe that 10.1% of samples collected in non-hospital areas were detectable for coronavirus, and 17.7% of hospital samples identified the presence of SARS-Cov-2. In addition, no viable viral samples could be found in 242 samples considered detectable (positive) for its presence.

Similarly, in an extensive survey involving 94 samples taken from the environment, the spread of COVID-19 throughout the environment was found to be irrelevant, with the exception of areas near patients infected with the virus. In total, it was possible to identify the presence of this virus in only 4% of the samples, all near hospitalized patients (Piana et al., 2021).

It is necessary, however, to recognize that the low number of samples performed in this research is far from providing a definitive conclusion on this issue, but that, together with the previously cited studies, help to provide a more solid set of data so that we can understand the mechanisms of virus transmission.

Similar to Piana et al. (2021) who searched for perdidotes (saliva sputum) and biofluids in their samples, the amplification of the human RNaseP gene in this study contributes to the collection of information by highlighting sanitation conditions and possible risk areas where there is a greater movement of people and on frequently touched surfaces. These results provide information on the cleaning conditions in areas frequented by the population of Cascavel-PR, who seek the services of the Physiotherapy, Dentistry, School Pharmacy and Outpatient Clinic (community vaccination point) besides favoring the prevention of cross-contamination of samples from LaBioqMol, where RT-PCR tests are performed for the diagnosis of infection by the SARS-Cov-2 virus, ensuring the reproducibility and reliability of the results.
Thus, it is necessary to emphasize the importance of maintaining cleanliness in these environments, with the use of soap and water for the removal of dust, secretions, or other organic matter, always followed by the use of some chemical disinfectant such as sodium hypochlorite or 70% alcohol (World Health Organization, 2020), which has been shown to be extremely effective (Gonçalves et al., 2021; Kanamori et al., 2020). The use of other preventive measures, such as the use of masks, constant hand sanitization, and social distancing, along with adequate ventilation of closed places, is also preached as essential to minimize virus transmission (Islam et al., 2020; Liu et al., 2020).

4 CONCLUSION

Although the present work did not show the presence of SARS-Cov-2 in the samples collected, the information obtained from this research shows a relevant role in guaranteeing an internal control of the tests performed in the Molecular Biochemistry Laboratory of Unioeste and other Healthcare environments, by avoiding cross contamination of the samples, and evaluating the effectiveness of the measures currently established to mitigate the spread of the virus in the healthcare areas of the Cascavel campus of the Universidade Estadual do Oeste do Paraná. This research also adds to the view that, in "real life" situations, the presence of SARS-CoV-2 material is less than thought at the beginning of the pandemic. Besides the social distancing and the mass vaccination of the world's population, simple measures, such as ventilation in the open air, reduction of crowds, constant cleaning of risk areas, and the use of personal protective equipment can be associated as an effective set to control the transmission of the virus. Certainly the world population will be more prepared if they encounter the same Public Health problem again in the future.
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