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# EXPERIENCE OF IMPLEMENTIG THE MOLECULAR DIAGNOSIS OF COVID-19 IN THE ADOLFO LUTZ INSTITUTE REGIONAL LABORATORY CENTER LOCATED IN PRESIDENTE PRUDENTE, SÃO PAULO

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**Abstract**: This study aims to describe the results of the implementation of the molecular diagnosis of Covid-19 at the Regional Laboratory Center Adolfo lutz Institute of Presidente Prudente (CLR IAL PP-V). This is a descriptive observational study carried out from April 2020 to June 2022. The implementation of the diagnosis was only possible from the "Term of Cooperation between the Municipality of P. Prudente and the Public Ministry of Labor - PRT/15th Region of P. Prudente, with the consent of the Health Department of the State of São Paulo". This term allocated financial resources for the acquisition of equipment, supplies and adaptation of the physical facilities of the laboratory. Additionally, P. Prudente's municipal civil servants were made available and employees were temporarily hired to assist in the laboratory routine. The laboratory for the diagnosis of Covid-19 was opened on August 13, 2020. From its implementation until June 2022, more than 113,000 diagnostic tests for Covid-19 were carried out. While in 2020, approximately 30% of the samples received were analyzed; in 2022, the laboratory performed analyzes of 95% of the total samples received. Carrying out this exam in the CLR IAL PP-V was also important in terms of reducing the time to release the results, which averaged 1.2 days in general from 2020 to 2022, not exceeding 2.5 days. The results of the present study demonstrate the importance of the work of the Adolfo Lutz Institute in the prevention, control and laboratory surveillance of Public Health problems, especially in controlling the spread of the pandemic within its region of coverage.

**Keywords**: Coronavirus; pandemic, SARSCoV-2, public health surveillance; laboratory surveillance.

## INTRODUCTION

Covid-19 (Coronavirus disease 2019) is a highly contagious communicable disease caused by a new coronavirus called SARSCoV-2 (Dutta et al., 2022). This viral infection is believed to have started with a zoonotic transfer from a seafood market in Wuhan, Hubei Province, China (Zhu et al., 2019). This virus then spread rapidly around the world, being considered by the World Health Organization as a global pandemic since March 2020 (Cucinotta; Vanelli 2020). Therefore, it is extremely important to use diagnostic methodologies to assist in the detection of the SARS-CoV-2 virus to prevent its spread, as well as to know the panorama of the spread of the disease.

Laboratory diagnosis by molecular biology is made by researching segments of the SARSCoV-2 viral genome in samples from patients suspected of the disease and performing the polymerase chain reaction in real time or quantitatively. (*reverse transcription quantitative polymerase chain reaction*, RT-qPCR). In this technique, target segments of the virus genome that encode conserved and specific regions of SARS- CoV-2 are searched (Caterino-de-Araujo, 2021).

The use of RT-qPCR provides important information in the early stages of infection, as it searches for the pathogen directly through the detection of its nucleic acid. Therefore, it allows early detection and differentiation from other viral respiratory infections, with high sensitivity and specificity (Menezes; Lima; Martinello, 2020). Despite being considered the most effective method of detection, it is noteworthy that a negative result in RT-qPCR does not completely rule out the possibility of infection by the virus, and it is recommended that the result be combined with clinical observations, patient history and epidemiological information. in the region (Nogueira; Silva, 2020).

It is worth mentioning that the Adolfo Lutz Institute, located in São Paulo, was the first laboratory to implement the diagnosis of Covid-19 by RT-qPCR in Brazil. The researchers from the Strategic Laboratory and the Virology Center, together with the Institute of Tropical Medicine of the Faculty of Medicine of the University of São Paulo, used the protocols recommended by the World Health Organization (Caterino- de Araujo, 2021) and performed the sequencing of the SARS-CoV-2 virus in less than 24 hours (de Jesus et al., 2020).

Currently, IAL has ten technical centers: Food; Bacteriology; contaminants; Immunology; Reference Materials; Medicines, Cosmetics and Sanitizing Products; Pathology; Interdisciplinary procedures; Parasitology and Mycology and Virology. In addition to the Central Laboratory, the Institute has twelve regional laboratories, strategically distributed throughout the state of São Paulo. Among these, the one located in Presidente Prudente, SP.

The Adolfo Lutz Institute Regional Laboratory Center of Presidente Prudente (CLR-IAL-PP-V), Central Laboratory of Public Health (LACEN) of São Paulo, is a reference in the diagnosis of diseases of epidemiological interest and serves 45 municipalities covered by the Regional Department of Health — DRS XI of Presidente Prudente, being the Epidemiological Surveillance Group (GVE) 21 of Presidente Prudente and GVE 22 of Presidente Venceslau. This laboratory was inaugurated on March 22, 1956 and since then has been working in the areas of Chemicals/Bromatology and Medical Biology, in addition to producing relevant knowledge for public health, developing applied research, promoting and disseminating scientific works, collaborating in the elaboration of standards techniques, standardizing diagnostic and analytical methods.

The objective of this work is to present the results of the implementation of the diagnosis of Covid-19 by the CLR-IAL-PP-V in the DRS XI region of Presidente Prudente.

## MATERIAL AND METHODS

The present study is descriptive and was carried out from May 2020 to June 2022. The technique of participant observation was used, since the authors participated in the process of planning strategies and actions to expand physical and technological capacity, human and operational of the CLR-IAL-PP-V.

To achieve the objective of implementing the molecular diagnosis of Covid-19, the main steps to be carried out were defined:

1) Acquisition of the thermocycler equipment for real-time PCR;
2) Acquisition of other equipment (PCR hoods, centrifuge, refrigerators, freezers, ultrafreezer) and other inputs;
3) Physical structuring for the adequacy of the available rooms to carry out the stages of the diagnosis;
4) Human resources trained to carry out the receipt and screening of biological samples suspected for Covid-19;
5) Human resources trained to carry out laboratory tests;
6) Technical training in the steps of extracting viral genetic material and amplification for performing RT-qPCR.

The implementation of the molecular diagnosis for Covid-19 in the CLR-IALPP-V was made possible through the financial resources obtained by the Term of Cooperation between the Municipality of Presidente Prudente and the Public Ministry of Labor-PRT/15th Region by Presidente Prudente, with the consent of the São Paulo State Health Department, published on May 8, 2020 (São Paulo, 2020a).

From its implementation, the RT-qPCR exam for Covid-19 is performed in a

unidirectional flow consisting of five steps: (1) receipt and verification of clinical samples; (2) extraction of viral genetic material; (3) amplification of genetic material in real-time PCR equipment; (4) analysis, interpretation of data and (5) release of results. These results were entered and released in the Laboratory Environment Manager (GAL), which provides the management of laboratory routines, analysis results and reporting.

The amount of laboratory tests performed during the period from September 2020 to June 2022 were obtained from management reports at the GAL. In order to evaluate the time of release of the results, the date of receipt of the biological samples in the performing laboratory until the release of the results was taken into account.

## RESULTS AND DISCUSSION

After the Term of Cooperation between the Municipality of Presidente Prudente and the Public Ministry of Labor-PRT/15th Region of P. Prudente, the process of price/ supplier surveys began in May 2020, followed by the purchase of equipment and supplies. The delivery of items purchased at CLR IAL PP-V was carried out in June and July 2020.

Initially, equipment was acquired to perform the tests, such as the thermocycler for real-time PCR, PCR Caps, Centrifuge, as well as equipment to ensure the thermostability of biological samples, ultrafreezers at –80°C and freezer at – 30°C. In addition to equipment and supplies, the rooms available within the laboratory were structurally adapted, including circulation flows, in order to avoid cross-contamination.

In addition, so that the diagnosis of Covid-19 could be carried out effectively, the Municipal Secretary of Presidente Prudente made available three servers to provide services at the CLR-IAL-PP-V and assist in the laboratory routine. Additionally, the Butantan Institute (IB) hired a professional during the years 2020 and 2021; and participation of service providers in the form of collaborators through Projects/ Operations subsidized by the Administrative Council of the Special Health Fund for Mass Immunization and Disease Control – FESIMA, linked to the project "Responses to the public health emergency due to Covid-19 in cases of severe acute respiratory syndrome and flu syndrome in sentinel units" for professionals, technicians and researchers, public servants or not, performed outside their regular working hours.

During this period, other challenges were presented in the process of implementing this diagnosis, such as the dispute between different countries in the world for the acquisition of inputs and equipment, given the need that everyone had to carry out the diagnosis of Covid-19. Among them, there is a lack of materials such as synthetic swabs (100% rayon) for collection, plastic materials (tips, cryotubes, reaction microplates, etc.) and supplies such as enzymes and probes used in real-time PCR (Silva et al., 2020).

The laboratory was enabled to participate in the REDELAB Covid-19 on August 13, 2020, and on this date the Multiuser PCR Laboratory was inaugurated at CLR-IALPP-V. After the employees carried out the technical training and validation of the realtime PCR method, the molecular diagnosis for Covid-19 began on September 10, 2020.

With the implementation of the Molecular Biology Laboratory for the diagnosis of Covid-19, the initial planning was to serve the 45 municipalities in the Presidente Prudente region. However, at that time, the laboratory's analytical capacity was approximately 92 tests/day, due to the manual extraction of the virus's genetic material using the Lucigen® and Bioclin® kits. In this sense, the main attribution of the IAL network was to prioritize samples of

suspected Covid-19 that were classified in the GAL as severe cases, deaths, outbreaks, and the CLR IAL PP-V also prioritized samples suspected of health professionals from all over the world. region and requisitions from the municipality of Presidente Prudente, due to the Cooperation Agreement. Requests from suspects classified as "Covid-19 - Flu Syndrome", which exceeded the analytical capacity per day, continued to be sent daily to the Adolfo Lutz Institute for redistribution in the Diagnostic Laboratory Platform in São Paulo, coordinated by the IB.

According to CIB Deliberation nº 55/2020 published on July 2, 2020 (São Paulo, 2020b), Covid-19 testing was extended to the entire population symptomatic of Influenza Syndrome. Due to the size of the Regional Department of Health (DRS) XI region in the state of São Paulo, having according to IBGE (2021) a population of 782,748 inhabitants, rapid diagnosis is of great importance for which surveillance actions, prevention and control are carried out efficiently.

Therefore, following the Deliberation, all patients suspected of Covid-19 must have samples collected by the Basic Health Units, Emergency Care Units, Covid Screening Centers, coordinated by the Municipal Epidemiological Surveillance and Hospitals in the region covered by the DRS XI, registered in the GAL as "Covid 19 Flu Syndrome" and forwarded to the CLR IAL PP-V. Then, the laboratory transported these requisitions/ samples to São Paulo-SP where these tests were performed by the Instituto Adolfo Lutz Central or by the Platform of diagnostic laboratories. Unfortunately, this redistribution procedure of requisitions/samples classified as "Influenza Syndrome" generated delays in carrying out the exams and releasing the results exceeded 72 hours of collection.

Since the beginning of the pandemic, scientists and technicians have worked on all fronts to develop new techniques and assays capable of detecting the SARS-CoV-2 virus (Nyaruaba et al., 2021). As shown in Tables 1 and 2, several commercially available protocols and kits were used in this laboratory 2020 with the installation of the Extracta-32, LOCCUS® equipment in loan with the IB, which increased the laboratory's analytical capacity to approximately 360 exams/day in January 2021.

On February 25, 2022, the automated MAELSTROM 9600, TANBEad® extraction for the diagnosis of Covid-19 by RT-qPCR during the period 2020 to 2022.

| Extraction type | Description | Provenance |
|---|---|---|
| Quick extraction | QuickExtract™ DNA Extraction Solution | Lucigen, USA |
| Manual extraction | Extraction of DNA/RNA viral BioGene | Bioclin, MG, Brazil |
| Automated extraction | Extracta Kit – RNA and DNA Viral (MVXA-P016FAST) | Loccus, SP, Brazil |
| | Extracta Kit Fast – DNA and Viral RNA (MVXA-P096 FAST) | Loccus, SP, Brazil |

Table 1: Description of viral genetic material extraction kits.

| Kit description | Provenance | Targets |
|---|---|---|
| Kit Molecular SARS-CoV-2 (E) | Bio-Manguinhos (Brazil) | E, RP |
| Kit Molecular SARS-CoV-2 (EDx) | Bio-Manguinhos (Brazil) | E, RP |
| Kit Biomol OneStep | IBMP (Brazil) | N, ORF 1ab |
| Allplex 2019-nCoV Assay | Seegene (Korea) | N, RdRP, E |
| Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex | Shangai ZJ Bio-Tech Co., Ltd (China) | N, ORF 1ab, E |

Table 2: Description of the amplification kits used in the assay of RT-qPCR.

| Year | Received samples (n) | Performed tests (n) | Percentage of exams performed (%) |
|---|---|---|---|
| **2020** | 19,851 | 5,930 | 29,9 |
| **2021** | 98,617 | 70,466 | 71,5 |
| **2022** | 41,760 | 36,773 | 94,7 |
| **TOTAL** | **160,228** | **113,169** | **70,6** |

Table 3: Production of samples received and RT-qPCR exams performed for SARS-CoV-2 research from September 2020 to June 2022.

2020: September to December

2021: January to December

The automated extraction of viral genetic material was implemented on December 29, equipment was installed, capable of running 96 samples every 30 minutes, increasing the analytical capacity to approximately 660 exams/day.

The Covid-19 diagnosis working group, which started in September 2020, has already surpassed the mark of 113,000 tests carried out by June 2022 (Table 3). While in 2020, 30% of the samples received were performed; in 2022, the laboratory performed 95% of the total samples received. Due to the Covid-19 Laboratory Network being coordinated by the IB, occasionally some of the biological samples suspected of Covid-19 were forwarded to the IB for diagnosis and sequencing. This justifies why the average of 100% of the total samples received was not reached.

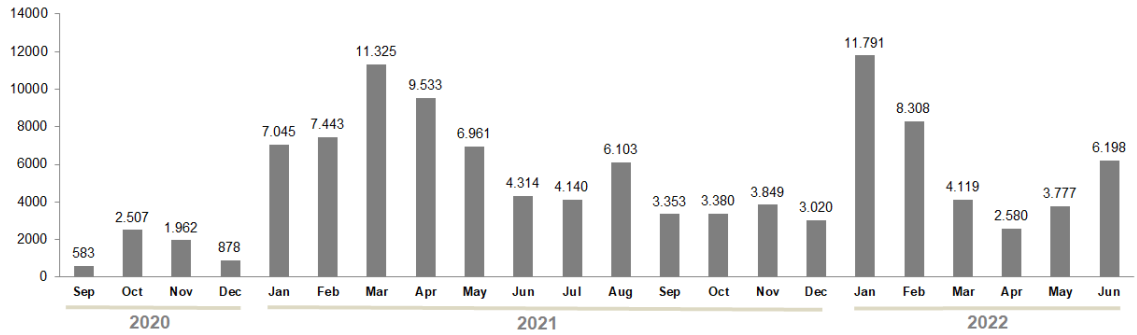


Figure 1. Total Covid-19 exams performed by CLR Adolfo Lutz Institute of Presidente Prudente in the period from 2020 to 2022.

Source: GAL System, Adolfo Lutz Institute.

According to figure 1, it is possible to visualize the monthly production of RT-qPCR exams for Covid-19 carried out in the CLR IAL PP-V since 2020. The first quarter of 2021 stands out, in which the laboratory performed more than 11,300 tests in March 2021. The growth in the number of tests performed for Covid-19 in March coincides with the emergence and spread of new variants of the SARS-CoV-2 virus around the world, including B.1.1.7. (Alpha), B.1.351 (Beta), P.1 (Gamma) and B.1.617.2 (Delta) (Benito et al., 2021; Zhan et al., 2022). These variants have higher transmissibility compared to

the original virus and the ability to increase disease severity in patients (Choi; Smith, 2021). The execution of this amount of exams was only possible with the readjustment of the working hours of the employees involved in the diagnosis and collaboration of the Other laboratory employees who were not directly involved.

In November 2021, a new variant B.1.1.529 (Omicron) of the SARS-CoV-2 virus was identified, which presented a greater number of mutations, responsible for its high transmissibility (Karim; Karim, 2021). The rapid spread of this variant is due to its ability to evade the immune system, being responsible for the infection of vaccinated and/or previously infected individuals by the coronavirus (Fan et al., 2022). The description of this new variant followed the increase in the number of exams performed in January 2022, with more than 11,700 exams/month. In this period, there was an increase of approximately 500% in the performance of exams, when compared to October 2020.

With the laboratory diagnosis of Covid-19 being carried out in Presidente Prudente, the release of results took an overall average of 1.2 days from 2020 to 2022, not exceeding 2.5 days (Figure 2).

Carrying out the trials and releasing the results within 72 hours enabled state and municipal surveillance to take measures to better cope with the pandemic, such as isolating patients without severe symptoms, early treatment of critically ill patients, as well as helping with clinical management in hospitals. referral hospitals, enabling the release of infirmary beds and Intensive Care Units, in case of negative results from patients with suspected Covid-19.

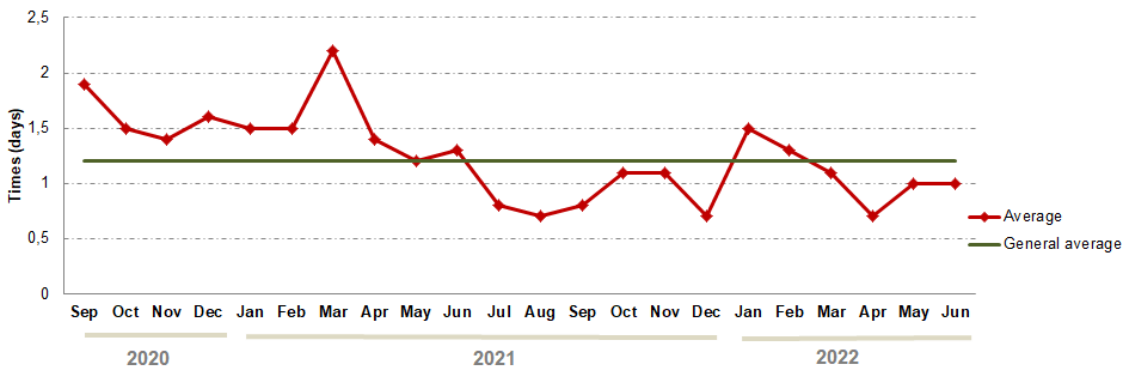


Figure 2: Release time (in days) of RT-qPCR results for Covid-19 on: CLR Adolfo Lutz Institute of President Prudente.

## CONCLUSION

The implementation of the Covid-19 diagnosis in the Presidente Prudente region demonstrated the importance of the Adolfo Lutz Institute in the prevention, control and surveillance of Public Health problems, especially when it comes to a pandemic.

In this sense, it was observed that rapid laboratory diagnosis was one of the most efficient strategies in the world to contain the advance of the spread of the SARS-CoV-2

virus, since, when having knowledge of confirmed cases, health surveillance actions can be carried out efficiently, in order to guarantee universal access and comprehensive care to users of the Unified Health System.

Therefore, it is important that scientific advances are constantly applied to laboratory surveillance systems with the development of efficient, rapid detection methods with accurate and reliable results; investments in state-of-the-art equipment/platforms and in the qualification of health professionals, so that they are prepared to face new problems or the emergence of other pandemics.

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