

ICB-USP begins tests to increase the capacity for a highly reliable and low-cost diagnosis of Covid-19 throughout the country

Increased testing, speedy return of results, applied throughout the country, high reliability, using domestic, low-cost reagents. These six advances in the fight against the novel coronavirus have come together in two Covid-19 tests developed by the Institute of Biomedical Sciences of the University of São Paulo (ICB-USP). Their efficacy has already been proven and they may be applied immediately.

The tests may be performed in small and medium-sized cities at a low cost and the results come back on the same day, which makes it possible for infected persons to receive the correct treatment and to be isolated as quickly as possible.

In order to develop these tests within a relatively short time span (they began to be studied back in March), ICB-USP researchers concentrated their efforts on adapting the equipment and methods previously used – to diagnose several other diseases – to diagnose Covid-19.

One of the tests is based on a polymerase chain reaction (PCR), which reveals whether or not patients are carrying the virus in their bodies. The other, focused on serological diagnosis and based on the ELISA method, shows whether or not the patient has been in contact with the novel coronavirus.

The ICB's PCR – The studies consisted of adapting the conventional PCR to Covid-19, which is able to diagnose numerous infectious diseases, such as measles, influenza, tuberculosis, and genetic problems. In the case of Covid-19, the test – named RT-PCR-ICB – analyzes secretions from the airways, in order to detect the presence of the novel coronavirus in the patient's body.

The new test ranks high in terms of reliability: 98% sensitivity (capacity to detect positive cases) and 100% specificity (capacity to exclude negative cases).

The RT-PCR-ICB test will be an alternative – with advantages – to one of the tests most used in Brazil to diagnose Covid-19: Real Time PCR. The studies were conducted by virologist Edison Luiz Durigon, who is the Coordinator of the Clinical and Molecular Virology Laboratory of ICB-USP.

One of the new things about this is that the RT-PCR-ICB will be done by equipment already in existence in most universities, hospitals and public and private laboratories located in numerous cities around the country. That equipment, called a thermal cycler, costs about R\$ 15,000. As for the Real Time PCR test, the equipment can cost as much as R\$ 150,000.

Another difference is in the cost of the reagents. The RT-PCR-ICB test uses reagents manufactured in Brazil, which are cheaper and can be delivered by the manufacturer within just a few days. Real Time PCR uses imported materials that, besides being expensive, take up to two months to arrive in Brazil.

One more difference: the available work force. Mid-level technicians and professionals with degrees in Biology or in Biomedical Sciences have the necessary knowledge for performing the RT-PCR-ICB tests. Real Time PCR can only be performed by professionals who are trained by the manufacturer of the thermal cycler, which implies the involvement of more time for training in that skill.

With these characteristics, Real Time is performed exclusively by large laboratories, which are concentrated in large cities. With the high demand for testing throughout the country, the results are delayed in coming back to the patients – and that can also cause delays in determining the proper treatment procedure.

There is also the cost differential for performing each test: about R\$ 15.00 for each RT-PCR-ICB, as against approximately R\$ 80.00 for each Real Time PCR.

The result of the RT-PCR-ICB takes about the same time as the Real Time PCR, or approximately six hours. “Both are equally effective, sensitive, and specific,” states Prof. Edison Luiz Durigon.

ELISA – The ICB also developed a serological test of the ELISA type (enzyme-linked immunosorbent assay) specifically for Covid-19, from antigens produced in their laboratories. Analyses of blood samples identify the presence of antibodies (IgA, IgM, and IgG) in patients, in order to discover if they are, or already had been, infected by the coronavirus, including asymptomatic cases. Its success rates are 92% for detecting positive cases (sensitivity) and 98% for excluding negative cases (specificity).

For this test, ICB-USP’s contribution to combatting the coronavirus was the laboratory production of SARS-CoV-2 proteins that react with the antibodies of infected patients.

Those proteins were generated in genetically modified bacteria to produce the purified proteins of the virus and, then, adapt them to the ELISA test. “The entire process required the participation of teams of three ICB laboratories having different specializations,” stated Professor Luís Carlos de Souza Ferreira, Director of the Institute and participant in the studies. “The methodology also is the fruit of partnerships with companies that work in conjunction with the ICB in the development of serological diagnosis kits similar to those developed for people who have been infected with the Zika and dengue fever viruses.”

The ELISA-ICB test offers advantages compared to similar tests, mainly with regard to the cost of each reaction (about R\$ 20, as against the amounts that could go as high as R\$500 at private laboratories) and the origin of the reagents (national, cheaper and available on the market, instead of imports, more expensive and delayed delivery).

The efficacy of the ELISA-ICB test was proven by a study in which 934 people were tested: professors, students and employees of the Institute. Of that total, 9.3% tested positive for IgG, that is, they were exposed to the virus and developed an immunity. On the other hand, 1.5% of the people tested positive for IgA, indicating recent exposure to the virus (*see the differences, below, between the types of antibodies*). “The test was created with two distinct SARs Cov-2 antigens (proteins N and S), and those who were confirmed for IgA also were given the molecular test (PCR) to identify the people who still had the virus in their organism,” Ferreira adds.

EXPANSION OF TESTING – Both tests developed by the ICB-USP can contribute to managing infections within the populace, in companies, public or private organizations, as well as in small and medium-size cities and municipalities.

A laboratory equipped for RT-PCR-ICB testing can perform around 100 tests per day; for the ELISA-ICB, about 500 tests per day. These figures reflect the reality of a city like Araraquara, with 260,000 inhabitants. Located in outstate São Paulo, Araraquara is outstanding for its fight against Covid-19, due to the number of tests it performs, above that which most cities do and, consequently, to its low death rate for the disease: 0.99%, as against the national average of 3.6%. The city of Araraquara currently requires 120 Real Time and 300 serological tests per day.

The tests developed by ICB-USP will bring benefits not only to Brazil, but also to countries that lack large laboratories and find it difficult to obtain the materials for Real

Time tests. “The conventional PCR technology has been in use for over 20 years; hospitals and universities, the world over, have access to it. The production of proteins for ELISA is not expensive and can be done in any country. The two methods that we have developed can easily be used in African, South American, or Caribbean countries,” states the ICB Director.

Types of ELISA tests:

IgA: Detects whether or not the individual has immunoglobulins (antibodies) within three to five days after being contaminated by the virus.

IgG: Detects whether or not the individual has antibodies against the coronavirus 14 days after being contaminated by the virus.

Comparisons between ICB-USP tests and those that are available on the domestic market:

<i>Test name</i>	<i>RT-PCR (ICB-USP)</i>	<i>Real Time RT-PCR</i>
Type	Molecular	Molecular
What it does	Detect whether or not the individual is contaminated by COVID-19	idem
Efficacy	98% sensitivity 100% specificity	idem
Time required for doing the test	6 hours	6 hours (Today, the result actually takes one week to be ready, due to the centralization of laboratories and the resulting waiting lists)
Laboratory capacity for performing the tests	Up to 100 tests/day	300 tests/day
Means of performing the test	Nasal swab	Nasal swab
Protocols	Already approved and released to the scientific community to be distributed to laboratories across the entire country	Complex protocols; needs training by the equipment manufacturer
Use by the laboratories	As of August 2020	Already in use

Cost of each test	R\$ 10.00 to R\$ 15.00	About R\$ 70.00
Cost of main equipment	About R\$ 15,000 (called a thermal cycler; common equipment and all of the States have laboratories equipped with it)	About R\$ 150,000 (called a thermal cycler for Real Time PCR, but few laboratories) have it
Origin of the reagents	National (their manufacture needs to be ordered, but they can be quickly readied, in up to three days)	Imported (today, it takes up to two months for delivery, due to the pandemic)
Level needed by the workforce for performing the test	Technician, or undergraduate degree in Biology or in Biomedical Sciences	Idem
Other differential	Replicability is easy for other developing countries	

Test name	ELISA (ICB-USP)	Similar ones on the market
Type	Serological	Serological
What it does	Detects antibodies, in order to know if the individual has been infected	idem
Efficacy	92% sensitivity 98% specificity	Variable. Very quick tests, in general, present very inferior performance
Time required for doing the test	3 hours	From 15 minutes to 3 hours, depending on the test (Today, the result of ELISA tests could take several days to be ready, due to the centralization of laboratories and the resulting waiting lists)
Laboratory capacity for performing the tests	500/day	Up to 1,000/day (in an automated laboratory, which are very few)
Means of performing the test	Blood sample	Blood sample
Protocols	Already approved and released to the scientific community	Already in use and known by the market
Use by the laboratories	August 2020	Already in use
Cost of each test	About R\$ 25.00 per reaction	Amounts vary between R\$ 100.00 and R\$ 500.00 (depending on the type of test)
Cost of main equipment	About R\$ 50,000 (spectrophotometer for plate reading)	Variable, according to the type of test
Origin of the reagents	National	Imported
Level needed by the workforce for performing the test	Technician, or undergraduate degree in Biology or in Biomedical Sciences	Idem

Other differentials

- By making the test more accessible, it will be possible to quickly diagnose the disease for adequate treatment and isolation of patients
- Replicability is easy for other developing countries

PRESS RELATIONS

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