Development of serological assays to detect SARS-CoV-2 antibodies

August 20 2020

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Approximately four months after the initial description of cases of atypical pneumonia in Wuhan, China, in December 2019, COVID-19 had become a major pandemic threat. By April 12, 2020, around half of

the world's population was in lockdown, with 1.8 million officially diagnosed cases. Scientists from the Institut Pasteur, the CNRS, Inserm and Université de Paris conducted a pilot study to evaluate the reliability of several laboratory tests with the aim of gaining a better understanding of the profile of antibody responses to SARS-CoV-2 and how the virus is spreading among the population.

Four tests for the detection of SARS-CoV-2 antibodies were developed and evaluated, as well as two tests for the detection of neutralizing antibodies. These tests, known as laboratory assays, are a first stage in epidemiological research on COVID-19. The results of this study were published online on medRxiv on April 24, 2020, then in *Science Translational Medicine* on August 17, 2020.

Evaluating the prevalence of asymptomatic and symptomatic cases of SARS-CoV-2 infection and their antibody response profile is a vital part of efforts to contain the outbreak and shed light on how the virus is spreading.

PCR tests are currently widely used in France and worldwide, including by the Institut Pasteur (the National Reference Center for Respiratory Viruses, hereafter referred to as the CNR), to diagnose COVID-19 and to detect and quantify SARS-CoV-2 RNA. These virological tests are essential in identifying and monitoring individuals with active infection.

A number of serological assays are also in use. There are two types:

- Antibody detection assays, which show whether an individual has developed antibodies against SARS-CoV-2 proteins and therefore previously contracted the virus.
- Neutralization assays, which determine whether an individual has neutralizing antibodies and is therefore immunized against the virus.

The importance of reliable tests

The reliability of these tests is crucial. Several teams from the Institut Pasteur, the CNRS, Inserm and Université de Paris therefore set out to develop serological assays, conducting a pilot study to evaluate the reliability of four detection assays by measuring levels of SARS-CoV-2 antibodies. They also developed two new detection assays, giving them an additional point of comparison. Groups of blood samples were taken from different categories of individuals. These groups, used to evaluate the assays, can be broken down as follows:

- samples from 400 pre-pandemic individuals (2017-2019), used as comparative samples to establish the specificity of the assays by ensuring that there were no or very few "false positives";
- samples from 51 COVID-19 patients with severe or critical forms, from Bichat Hospital in Paris. These samples determined the sensitivity of the assays and enabled the scientists to study the kinetics of antibody response;
- samples from 209 individuals with mild symptoms (such as fever or cough) taken in the Oise département on March 3 and 4, 2020;
- samples from 200 asymptomatic blood donors in Oise taken between March 20 and 24, 2020.

Seroprevalence results

Seropositivity (the presence of antibodies) was detected in 32% of individuals with mild signs compatible with COVID-19 in the 15 days before the samples were taken, and in 3% of asymptomatic blood donors.

Antibody response time

The scientists determined the antibody response time in hospitalized individuals with COVID-19. Antibodies appeared 5-6 days after the first symptoms, with neutralizing activity after 7-14 days.

This timeline is probably longer in mildly symptomatic or asymptomatic individuals, and the antibody titers (concentration) are probably lower.

Description and evaluation of the assays

The research teams designed four laboratory assays to evaluate SARS-CoV-2 antibody levels in human serum.

ELISA assays: The two ELISA assays are conventional assays based on the full-length N protein of SARS-CoV-2 (ELISA N) or the extracellular domain of the virus spike protein (S).

Advantages: these assays are based on a technique that can be easily transposed and is used in commercial kits. The ELISA S assay was slightly more sensitive than the ELISA N assay.

For both assays, the question of how they might be used on a large scale or brought to market was raised, since they are currently in-house tests. Assays based on a similar principle have already been marketed and are currently under evaluation by the CNR.

The performance of commercial assays is evaluated by the CNR using serum samples determined as negative or positive for SARS-CoV-2 antibodies on the basis of at least two of the serological assays developed at the Institut Pasteur.

S-Flow assay: This assay detects the SARS-CoV-2 spike protein in its natural conformation, at the cell surface. It is highly sensitive and specific. The results were similar to ELISA S, with higher sensitivity for

low antibody levels. But the assay requires specialist equipment (a flow cytometer) that is less widely available than the plate readers generally used in medical laboratories. This makes it more suitable for use in epidemiological research rather than large-scale diagnostic testing.

LIPS assay: This "immunoprecipitation" assay uses a different technique from the other tests and detects antibodies binding to SARS-CoV-2 N or S proteins or their subdomains. The test provides detailed profiling of viral protein regions targeted by the antibody response. The assay therefore uses various "target antigens," and the sensitivity of the test varies depending on the antigen detected. For this study, part of N and part of S (S1) were used. This assay can also be used in its current form in most animals.

The research teams also developed two assays to detect neutralizing antibodies in serum samples from infected individuals, one using the infectious SARS-CoV-2 virus and requiring access to a BSL3 laboratory, and one based on a "pseudovirus" that can be used without the need for a BSL3 facility.

The neutralization assay with infectious SARS-CoV-2 is more complicated to implement as the virus needs to be handled in a BSL3 laboratory. It uses a fixed dose of the virus which destroys 100% of cells. After mixing it with different serum dilutions, the scientists observe whether the presence of SARS-CoV-2 antibodies is sufficient to neutralize the virus and inhibit viral multiplication, thereby preventing cell destruction.

The research teams also developed a neutralization assay known as Lenti S, based on a non-infectious pseudovirus instead of the SARS-CoV-2 virus. This assay does not require containment in a biosafety laboratory, making it easy to implement on a large scale.

Unlike ELISA-type assays, which detect antibodies, a neutralization assay measures the capability of antibodies to block entry of the virus into cells. It can therefore determine whether an individual has antibodies capable of limiting virus multiplication and likely to confer protection against further SARS-CoV-2 infection. The research teams are keen to adapt this assay so that it can be used for high-throughput testing. Further research will be needed to determine the quantity of neutralizing antibodies likely to confer protection and their persistence over time.

Correlation between the assays

To evaluate the comparative reliability of the assays, the research teams compared them by using three cohorts.

In hospitalized patients, a similar number of positive cases was obtained with the serological assays based on N and S; diagnostic application of the assay to detect anti-S1 antibodies was less sensitive, the aim of the latter being to investigate a correlation with protection.

With the cohort of symptomatic individuals from Oise, the S-Flow and ELISA S assays and the LIPS N+S1 combination provided very similar results and higher detection levels than with the other tests. In the blood donors, positive cases were only detected with the S-Flow and ELISA S assays.

In conclusion:

1. The research teams compared the performances of four SARS-CoV-2 antibody detection assays. In general they worked very well, with differences in sensitivity depending on the test and especially the antigens targeted.

2. With regard to the neutralizing antibody detection assays, the pseudovirus neutralization assay is simple and robust but it requires cell culture facilities and special equipment. Serological assays are used to estimate the level of antibodies binding to the virus rather than anticipate their functionality.

3. The scientists are currently establishing correlations so that they can estimate which blood samples have neutralizing capacity based on the levels of antibodies binding to different parts of the virus.

4. The presence of neutralizing antibodies in the blood is likely to indicate protection against further infection, particularly if the titer is high, but this has not yet been formally demonstrated.

5. Serological tests can be used in epidemiological research to determine seroprevalence among specific groups.

6. There was significant circulation of the virus within the cluster of first cases in Oise: 32% of people with moderate clinical signs compatible with COVID-19 had antibodies.

7. Results of the tests performed on asymptomatic individuals depended on the groups studied and their location. For example, out of the 200 tests performed on asymptomatic blood donors in Oise (individuals exhibiting recent signs are not permitted to give blood), 3% were positive.

"These assays are used to detect seroprevalence in epidemiological studies and also to diagnose individuals. They are also very useful for fully characterizing serum panels when evaluating commercial tests. This study involved several teams from the Institut Pasteur, working in cooperation with physicians and virologists from the Paris Public Hospital Network (AP-HP) and Inserm, epidemiologists and the French

Blood Service (EFS). We would like to think the patients and volunteers who donated blood for this study," emphasize the study's co-authors Marc Eloit, Hugo Mouquet, Olivier Schwartz and Sylvie van der Werf.

"The ELISA N and ELISA S assays are of particular interest in the current context. At the Institut Pasteur, thousands of tests could be carried out each week and the technique could be shared with other laboratories," explains Sylvie van der Werf, Head of the National Reference Center for Respiratory Viruses and joint last author of the study.

"LIPS-type assays have the same sensitivity as ELISA-type assays for the same antigen and are useful for studying in detail the probability of protection against further infection in cohorts of several thousand serum samples to determine which antigens are optimal for high-throughput testing – an approach that will be crucial in managing the easing of lockdown measures. Since this work , we have also extended the use of the LIPS assay to the detection of the infection by seasonal coronaviruses and their role regarding the protection or facilitation regarding SARS-CoV-2 infection," says Marc Eloit, Head of the Pathogen Discovery Laboratory and joint last author of the study.

"Since the beginning of the study in March 2020, the S-Flow assay has been used to study the extent of the humoral response in PCR+ individuals with mild symptoms. We are currently assessing the duration of this response. We are analyzing samples from convalescent individuals collected at different time points post symptom onset" says Olivier Schwartz, Head of the Virus & immunity Unit and joint last author of the study.

More information: Ludivine Grzelak et al. A comparison of four serological assays for detecting anti-SARS-CoV-2 antibodies in human serum samples from different populations, *Science Translational*

Medical Xpress

Medicine (2020). DOI: 10.1126/scitranslmed.abc3103

www.medrxiv.org/content/10.110 … 020.04.21.20068858v1

Provided by Pasteur Institute

Citation: Development of serological assays to detect SARS-CoV-2 antibodies (2020, August 20) retrieved 26 April 2024 from https://medicalxpress.com/news/2020-08-serological-assays-sars-cov-antibodies.html

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