



## Are you wondering if you have antibodies to COVID-19?

MUDr. Petra Havlíčková, Ing. Ivana Kubátová, Ph.D., Michal Horáček, MBA, PMP

*At the end of 2019, the first cases of pneumonia of unclear origin were described in Wuchan, the Central China's most populated city. During a short period of time, a new disease, later called COV/O-19, caused by a previously undescribed SARS-CoV-2 coronavirus, spread worldwide. On 11 March 2020, the WHO declared the spread of coronavirus a pandemic. The first case of the disease in the Czech Republic was registered on 1 March 2020 [1].*

The disease incidence of SARS-CoV-2 in the world and also in the Czech Republic is still increasing, as is the incidence rate [weekly number of newly infected people per 100,000 inhabitants] [2].

Many questions about COVID-19 are still unclear for the time being, for example in terms of the long-term consequences for the organism after the suffered disease, etc., time will show. Nowadays, it is important to observe hygiene measures, to inform the public about the importance of vaccination, but also to be aware of the basic immunological principles of the behavior of viral infections, which are

somewhat forgotten. Related to this is the detection of antibodies to COVID-19, which has been neglected so far, but stormily debated.

### Principles of antibody testing

The basic manifestation of adaptive immunity is the production of specific antibodies that can be detected. The presence of antibodies is a diagnostic criterion confirming the presence of a viral disease and often immunity to the disease as well. This thesis has been applied in practice for a long time and is taken for granted, but with the arrival of COVID-19 it seems as if it never existed. Unfortunately, the importance of measuring antibody levels is being questioned, both among the general public and professionals.

Long term immune response is ensured by memory B and T lymphocytes. B cells and plasmacytes are involved in production of antibodies while Th2 cells promote their production and Thc (cytotoxic T lymphocytes) kill infected cells [3].

During the course of infection, not just one type of antibody is produced, but several types directed against different viral antigens. Most important are antibodies

against spike protein [S or SI subunit], receptor binding domain [RBD] and nucleocapsid protein (N). During viral infection, different isotypes of antibodies (IgA, IgM, IgG) are formed.

After viral infection, IgA antibodies first appear in the blood. In the course of 3 to 7 days after the onset of symptoms, IgM antibodies are produced and with a 10 to 14 day delay our body produces IgG antibodies. Gradually, as the patient recovers, the level of IgM antibodies decreases, whereas the level of IgG rises. The IgG antibodies persist for a long time and are therefore an indicator of long-term immunity.

There is a huge debate about setting a threshold for the amount of antibodies that will be protective for the organism. The ECDC (European Centre for Disease Prevention and Control) considers the presence of neutralizing antibodies against



SARS-CoV-2 to be reliable evidence of protection, which is determined by the so-called "virus neutralization test" (VNT), a functional in vitro test that determines whether the given serum (or the antibodies present in it) protects the cell culture against viral infection [4]. If there are neutralizing antibodies in the serum, then the cells will remain alive because the virus will not multiply. If no antibodies are present, the virus invades the cells, multiplies in them and the cells die [5].

The VNT result correlates best with the presence of IgG antibodies against S1 or RBD antigens. Neutralizing antibodies are detectable on days 7 to 15 after the disease onset. They reach a maximum on days 14 to 22 of the disease. Later the level of these antibodies decreases slightly but remains at certain patient-specific levels for a long time. If someone has a repeatedly positive VNT result, they can be considered immune in the long term for approximately one year. Since VNT is not available to all patients, standard laboratory tests can be applied to determine the antibody levels [3].

In immunochromatographic methods, an antibody present in the sample under study reacts with a conjugate to form a specific immunocomplex (antigen-conjugate),

which migrates towards a specific protein immobilized on a porous membrane (nitrocellulose) in the form of a band. If an antibody is present in the sample, a specific reaction will occur at the flow site of the immunocomplex and the immobilized protein, signaling a positive result. The sample continues to flow through the membrane (due to the capillary effect) and binds to another immobilized protein, thus creating another reaction. In this way it is possible to combine several reactions (e.g. IgM, IgG, IgA and others).

Indicators or markers are the substances that are bound to one component of the reaction, it can be a labelled antibody or a labelled antigen. They serve for qualitative determination (or are also used for quantitative qualification in some tests). The most commonly used are enzymes, substances with fluorescent or luminescent nature, enzymes that help the formation of substances causing luminescence. In the case of LOMINA antibody tests, colloidal gold is used for the reaction, which has the advantage of allowing the reaction to be visible to the naked eye in the chemical bond space in the form of a coloured band. [6].

### Antibody testing- yes or no?

We have also seen great differences of opinion on the issue of vaccination. Most discussed was the recommendation of the Ministry of Health to vaccinate persons quarantine. However, this approach again contradicts the principles of antiviral immunity that have been used for many years. If we stimulate immunity with a vaccine immediately after an infection, thus at a time when specific immunity is still developing, then too much activation of the immune system can be dangerous. Considering the results of the studies so far, it would be good to start accepting that naturally induced immunity after COVID-19 infection provides long-term protection and that people after infection also have a significant contribution to collective immunity as vaccinated persons.

Of the studies available to date, the determination of antibodies to the coronavirus is a reliable method of demonstrating a history of COVID-19 infection, as reported in the studies below. In their study, Wang et al. examined 63 individuals on a cohort of subjects with an increase in neutralizing antibodies following COVID-19 infection. These patients were tested 1.3, 6.2 and 12 months after



infection. Of these patients, 41% were also vaccinated with the mRNA vaccine type. In patients who did not receive vaccination, both the reactivity of antibodies against RBD SARS-CoV-2 and the neutralizing activity remained relatively stable between 6 and 12 months after infection. Furthermore, the study results suggest that immunity in convalescent individuals will be very long and that convalescent individuals who receive the available mRNA vaccines will produce antibodies and memory B cells that should be protective against circulating SARS-CoV-2 variants. [7]

In their study, Turner et al. addressed the issue of the spread of infection in persons who had undergone SARS CoV-2. Results showed that in individuals who had mild SARS-CoV-2 infection (n = 77), serum anti-SARS-CoV-2 protein (S) antibody levels declined rapidly in the first 4 months after infection and then declined more slowly over the following 7 months. However, antibodies were still detectable at least 11 months after infection. Overall, the results suggest that even mild SARS-CoV-2

infection indicates robust antigen-specific, long-term immune memory in individuals. [8]. According to available data, antibodies are present in the blood for a long period of time (min. 10 months) and provide protection even in case of repeated contact with the infection, so much so that the eventual reinfection will have in most cases only a mild course or will be asymptomatic. Commonly available antibody detection methods reliably predict the results of the VNT, which according to the ECDC is the gold standard for immunity determination. In view of the above, the question arises as to why more emphasis is not put on the determination of antibodies. Knowledge of their levels can help us in many ways. For example, if you are deciding whether to be vaccinated after having COVID-19 infection or if you are wondering whether your body was able to make antibodies at all, either after vaccination or after suffering COVID-19.

An article by the National Institute of Public Health states that "According to the data available so far, 10-50% of people are

*reported asymptomatic courses of COVID-19, i.e. no clinical symptoms are present and only testing demonstrates the presence of the virus" [1] - whether it is the acute presence of the virus or antibody levels after a suffered disease. Whether I have antibodies or not is therefore very important information, as Reiglová, E., who interviewed immunologist Jiří Šinkora, writes in her article. He said, "When people are vaccinated during or just after an infection they're going through, they get emboli, myocarditis, etc. The immune system has not yet fallen asleep after the infection, and in another way, a powerful vaccine is pumped into it to trigger an immune system storm. There occur clots, clogging of blood vessels, inflammation of the heart, all in those people who were vaccinated too early. It's a crazy thing, but it's being done because the state hasn't told people to get a PCR test and an antibody test before they get vaccinated."*

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