

## SARS-CoV-2 (COVID-19) Screening and Detection

### Suggested protocol of therapy

Regarding the current situation caused by COVID-19, when the production and work becomes restarted, the need will arise for monitoring whether the workers, customers and clients

1. are already fought off the virus and not infectious anymore;
2. have not caught the virus, therefore they are not infectious;
3. may be symptomless when the examination takes place, but the virus is present and active in their body.

**Currently, there are five different methods to detect the infection caused by the virus.**

#### **1. rtPCR, which can confirm the presence of the RNA (genetic material) of the SARS-CoV-2.**

Specimen: nasal and/or pharyngeal mucosa cells; virus transport medium is used.

This is the reliable method for confirming the presence of the virus in the body; a positive result refers to this fact. A negative result likely refers to the absence of the virus, however, it is possible that the pathogen was not present in the sample because the specimen collection took place at the wrong time, involved no cells containing the virus, or was performed improperly. Certain references say that the rate of failure may reach 20–30%. Moreover, a positive result could occur even if the virus is not present in the body anymore, because there might be leftover RNA fragments near the cells which were infected before. The viral nucleic acid (RNA) can be detected in a few hours after the onset of the infection and be present for 10–14 days after the symptoms have ended. It can occasionally disappear, but can often reappear.

#### **2. SARS-CoV-2 antigen detection from nasopharyngeal smear**

Specimen: nasal and/or pharyngeal smear from virus transport medium.

This method can confirm or exclude the presence of the virus, too, detecting the surface proteins of the virus itself. Its tremendous advantage is that no complicated laboratorial background need be used. Its sensitivity is most likely lower than that of PCR. Since this method appeared merely a couple of weeks ago, we have not had much experience regarding this technic.

#### **3. Detecting IgG and IgM antibodies produced to the SARS-CoV-2 with lateral flow immunochromatography**

Specimen: capillary blood (a drop from finger prick), venous blood (whole blood or plasma). During the disease caused by the virus, the body produces immunoglobulins. From these, IgM appears earlier (5–8 days after the onset); it peaks around on the 14<sup>th</sup> day and disappears on the 21–28<sup>th</sup> day. IgG, on the other hand, starts being produced 14–21 days after the onset, peaks around on the 28<sup>th</sup> day. According to our current knowledge, it is present in the body after the disease, for weeks or months. The advantages of this method are its speed and the fact that if the result is negative, the probability of the absence of the antibody produced to the virus is 98–99%. Its disadvantage is that as long as the prevalence of the virus is low (very few people have actually been infected), there are more false IgM positives than real ones. Thus, in case of a positive IgM result it is more likely that the

antibody is present because of another disease (such as autoimmune disorders) than because of the virus itself. Applying a laboratorial (microbiological) evaluation enhances the efficiency of the test, especially if there are products of different manufacturers are available. Therefore, we consider using venous blood, laboratorial environment and the supervision of specialists more reliable than using finger prick blood during a point-of-care examination.

#### **4. Detecting IgG antibodies produced to the SARS-CoV-2 with immunoassay**

Specimen: venous blood (whole blood or plasma sample).

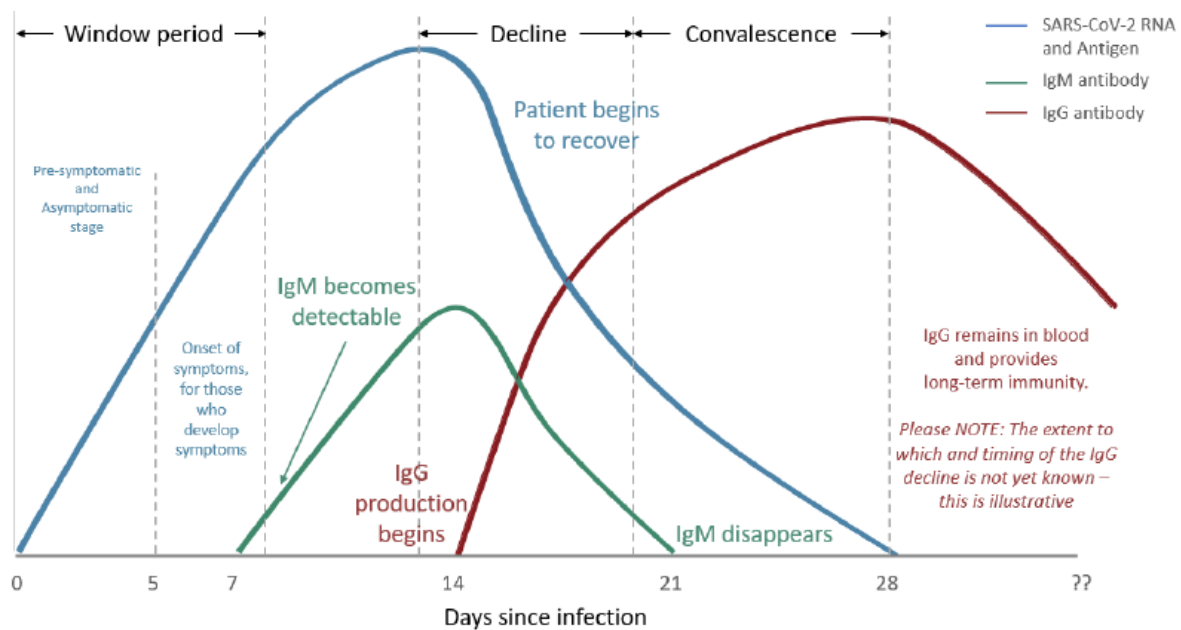
With this method the elevated amount of IgG immunoglobulin produced by the body during the disease caused by the virus can be detected specifically. IgG starts being produced 14–21 days after the onset, peaks around on the 28<sup>th</sup> day. According to our current knowledge, it is present in the body after the disease, for weeks or months. Its advantages are reliability and repeatability. Laboratorial environment, including a special immunoassay instrument must be used, though.

#### **5. Quantitative evaluation of IgG antibodies produced to the SARS-CoV-2 with immunoassay**

Specimen: venous blood (whole blood or plasma sample).

The level of IgG immunoglobulin produced by the body during the disease caused by the virus can be measured with high accuracy. With this method, the quantity of the elevated IgG can be evaluated, and it is possible to examine plasma pairs, too (specimen collected from the same person with a 1 or 2 weeks of time difference). If the level of IgG is rising, the patient is still in the active phase of the disease. If it is constant or slightly falling, it refers to the convalescent phase or to the fact that the disease ended. This method is capable of finding suitable IgG donors, too.

What our present information, using all the basic knowledge of the fields of virology, microbiology and immunology, regarding the possibilities of laboratorial diagnostics and the timeline of the disease is, is shown on the following diagram:



\*Disclaimer: This chart is for illustrative purposes only

Reference: [worldbank.org](http://documents.worldbank.org/curated/en/145161586536712080/pdf/Purposeand-Options-for-Testing-for-SARS-Cov2-the-COVID-19-Virus-Considerations-for-World-Bank-Task-Teams-Managing-COVID-19-Fast-Track-Facility-Operations.pdf)

(<http://documents.worldbank.org/curated/en/145161586536712080/pdf/Purposeand-Options-for-Testing-for-SARS-Cov2-the-COVID-19-Virus-Considerations-for-World-Bank-Task-Teams-Managing-COVID-19-Fast-Track-Facility-Operations.pdf>)

The blue line refers to the presence of the virus itself, which rises, obviously, first, which can be detected by rtPCR or direct viral antigen detecting tests (Method 1. and 2.).

The green line shows the start and the finish of the IgM production; this can be followed by IgM/IgG immunochromatography (Method 3.).

The dark red line shows the production of IgG. We can both detect the production itself (Method 4.), or define the quantity of the antibody (Method 5.).

The summary above clarifies that there is no method available with a specificity and sensitivity of 100%. Thus, we have tried to establish a protocol, applying different diagnostic methods to minimize the risks. Our laboratory is to use the best method(s) available, regarding specific questions.

The optimized protocol can consist of different steps, considering the goals and the financial possibilities.

Version A – suitable for screening the employees and establishing a potentially safe work environment

- a) filling out the coronavirus survey; the examination can be performed on people having been symptomless for 14 days
- b) serologic test (IgG and IgM detection) (Method 3.)
- c) for IgG positive persons we recommend quantitative evaluation, plasma pairs may be used, too (Method 5.)
- d) for IgM positive persons we recommend immediate quarantization. The forthcoming steps are determined by the epidemiological authorities.

In case of an IgM positive result not confirmed by either virus detecting method (PCR or antigen), the IgM/IgG examination must be repeated 14 days later. If the subject is still symptomless, IgM positive but the virus cannot be detected, we recommend immunological examination because the antibody positive result may refer to some other disease (such as autoimmune disorders).

*Only those can return to work who are*  
serologically negative (both IgG and IgM negative);  
or IgG positive and IgM negative;  
or IgM positive and the epidemiological authorities confirmed that they are able and allowed to work.

Version B – suitable for screening patients and pre-screening healthcare service clients (e.g. patients are to be treated)

a) filling out the coronavirus survey; the examination can be performed on people having been symptomless for 14 days

b) serologic test (IgG and IgM detection) (Method 3.) AND rtPCR (Method 1.) OR SARS-CoV-2 antigen test (Method 2.) negative results.

*Only those can be treated or use a service who are* IgG positive AND SARS-CoV-2 antigen negative; or IgG/IgM negative AND virus detection result (PCR or antigen method) is negative, too.

Regarding either method the frequency of repeating the tests may be a concern. Any examination can give information up to the appointment of the test made; in case of serological tests up to the “window period” before the specimen has been collected. This means, it is possible that the subject will have been infected by the time the result has obtained – therefore, the protocol above can significantly lower the risk of infection, but it is impossible to exclude it totally. Considering the current low prevalence rate, repeating the examination in every week or every two weeks, it appears to be safe to keep the cases of workplace infections at a low rate.

#### IMPORTANT!

The information available on the novel coronavirus has been broadening and changing every day. Therefore, any information on the testing methods is a constant subject of change. This text summarizes everything of our best knowledge, available information and data.

However, it cannot be excluded that anything stated here will change in a couple of days. In case of new data and information appearing, we take every reasonable effort to amend this text to make it up-to-date.

2<sup>nd</sup> of May, 2020, Budapest

A handwritten signature in black ink, appearing to read "Attila Bezzegh".

**Attila Bezzegh MD**

Professional Director

SmartBioma Ltd

## Annex

The novel coronavirus infection (COVID-19) questionnaire

- Have you ever had a positive coronavirus test (SARS-CoV-2 virus or antibody)?
- Have you had an illness of high temperature in the last 3 weeks?
- Has there been a person with novel coronavirus infection in your close environment?
- Have you experienced any of the following symptoms in the last 3 weeks?
  - loss of scent and/or tasting
  - severe pain in the limbs and headache which lasted for several days with recurring phases, too
  - unexplainable fatigue, sleepiness, weakness, immobilization (being unable to wake up) for several days
  - hematoma (bluish discoloration) on the limbs, especially on the toes, appearing simultaneously
  - catarrh of the upper respiratory tract, paranasal sinus inflammation, common cold AND pain in the head and the legs
- In case of the symptoms above, have you reported them to your family doctor, and/or have there been a thorough examination initiated?