

INDICATIONS OF ANTIBODY-BASED SEROLOGICAL FOR CLINICAL AND PUBLIC HEALTH MANAGEMENT OF COVID-19 INFECTIONS

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1. Summary indications serological tests

No change in international guidelines for use of serology tests and few further insights in immune response.

No new information on seroconversion kinetics, studies are currently looking into the possibility for using SARS-CoV-2 antibodies as a proxy for the neutralizing antibodies. However, there is still too much uncertainty to implement serological testing as a proxy for immunity.

As described in the advice of the RAG in April and May, serology tests are still recommended for some specific diagnostic indications and for epidemiological studies.

Serological surveillance is of critical public health importance to monitor SARS-CoV-2 infection prevalence and the eventual development of herd immunity.

Self-test is also part of this advice. Their role in diagnostic is not validated.

After having reviewed the literature, the RAG therefore assesses that

- There are still many knowledge gaps regarding the dynamics of the immune response,
- The best method to make a diagnosis of COVID-19 (and to assess infectiousness¹) remains the molecular technique, that directly targets the pathogen,

The indications of serology for diagnostic purposes remain limited but some additional indications are proposed (in blue):

1. For hospitalized patients with a suggestive clinical picture and in case of divergence between PCR and CT Scan, by paired sera since the (5-)7th day of illness and a second one at least 7-10 days later (for both IgM and IgG). Serologic testing can also support diagnosis of acute COVID-19 illness for persons who present late, only one a serum on 9–14 days after illness onset .
 2. For ambulatory patients with a suggestive and prolonged clinical picture but a negative PCR or who would not have benefited from PCR within 7 days after the onset of symptoms (Also by rapid test by first line care if tests validated on capillary blood).
 3. For differential diagnosis of atypical clinical presentation or to help support a diagnosis when patients present with late complications of COVID-19 illness.
 4. Serological tests could be useful in the monitoring of plasma from convalescent donors in a context of anti-COVID-19 therapy.
 5. In risk management in nursing home or other collectivities, a serology can help in interpreting a PCR positivity with high Ct-values/low viral load, surely in case of testing/screening of asymptomatic persons.
 6. Serological testing can be part of recognition of occupational diseases in the context of occupational medicine (but should not be charged to the clinical biology budget), but limited.
 7. Serological tests can be used to conduct epidemiological studies
- The use of (rapid) antibody-based serological assays for diagnostic self-testing is **not** appropriate because production of antibodies is delayed and does not allow immediate action (isolation/contact tracing).
 - A member of the RAG is nevertheless mentioning that the availability of such a test could attract the public into a public health important topic by allowing asymptomatic seronegative person to do repetitive serological testing in order to detect seroconversions. It is maybe more affordable options for certain type of organisations (ex.: sportclub, ...) also taking into account with possible shortage for PCR.

This proposition is done based on a review of knowledge in August 2020. Evidences remain weak. The RAG will continue to gather new evidence and will consequently adapt the recommendations.

¹ To be interpreted with caution in case of low viral loads/high Ct-values.

2. Introduction

In the context of COVID-19 crisis management, indications for the use of a diagnostic capacity may allow

- To diagnose a patient with symptoms based on the principle that the test must have an added value for the patient's health,
- To early confirm a diagnosis making possible to isolate patients and identify contacts in order to interrupt the chains of transmission,
- To describe the susceptibility of the population and to improve knowledge about the impact of the epidemic by performing epidemiological studies in support to the decision making process.

Indications for the use of diagnostic tests must take into account the principles of

- medical care: to allow a good therapeutic management
- the characteristics of diagnostic tests in microbiology: sensitivity, specificity, etc. taking the prevalence into account.

The recommendations contained in this advice are based on the best current state of knowledge but will need to be reviewed in the light of new findings, the evolution of the epidemic and the quality of available tests.

Serological tests measure the presence of antibodies.

They are eligible for use in the control of the COVID-19 epidemic as a complement to molecular tests (which directly demonstrate the presence of the pathogen), but their use remains limited by gaps in knowledge.

3. Objective

The RAG gave advices on the indications of serological tests on 05/05/2020 and 14/04/2020.

In the context of a long term strategy for the coming months, the RAG is requested to give an update of the previous advices.

4. Method

Literature review

International guidelines

Experts opinion

5. Summary of evidence having sustained the justification of indications

5.1. INFECTING DOSE OF SARS-COV-2 AND DOSE TRIGGERING AN ANTIBODY RESPONSE

Still unknown.

5.2. APPEARANCE OF ANTIBODY RESPONSE

The IgM and IgG antibodies to SARS-CoV-2 develop **between 6–15 days after symptom onset** [7-12]. The **median** seroconversion time for total antibodies, IgM and then IgG were day-11, day-12 and day-14 post symptom onset, respectively. The presence of antibodies was detected in <40% among patients within 1 week from onset, and rapidly increased to 100% (total antibodies), 94.3% (IgM) and 79.8% (IgG) from day-15 after onset [13]. IgM and/or IgG production is detectable in the majority of symptomatic patients **between 7 and 11 days** after the onset of symptoms.

IgM production may occur earlier, around day 5, but is frequently almost **concomitant** with IgG production.

Antibody levels appear to be higher in more severe cases but their kinetic seems to be delayed in such cases. Some studies are mentioning that some individuals with a confirmed diagnosis by RT-PCR may have weak, delayed or no antibody responses while a publication on a series of 285 hospitalized patients with COVID-19 highlights that all subjects were IgG seropositive after 19 days.

IgM and/or IgG production kinetics are still poorly characterized in asymptomatic or paucisymptomatic (mildly diseased) patients. In addition, one study showed that humoral response may be short-lived in patients with mild/no symptoms. A German study in health care workers looked at the immune response after a COVID 19 outbreak. Immune response after COVID-19 increases significantly over time but still approximately 22 % of COVID-19 patients did not mount a measurable serological immune response within 60 days. Exposed co-workers did not develop any relevant antibody levels at all.

This supports the conclusion that immunity after infection increases over time, but the antibody response does not develop reliably in all infected people, especially in asymptomatic individuals.

5.3. PROTECTION AGAINST CONTAGIOUSNESS

Serological tests do not make it possible to decide whether a person is contagious or not because seroconversion is not immediately accompanied by a simultaneous drop in viral load. There is no established correlation between the production of antibodies and the presence of the infectious virus while results of animal studies show however a critical role of neutralizing antibodies on viral excretion.

Some studies showed that patients can remain RNA-positive for long period of time despite presence of antibodies. Presence of RNA does not imply that the virus is viable. Infectiousness of these patients is unknown (44).

5.4. PROTECTION AGAINST REINFECTION

Usually, the protection against reinfection is depending of the persistence of the antibody response and the level of antibodies giving a protection.

The presence of anti-SARS-CoV-2 antibodies signifies an immune response developed from symptomatic or asymptomatic/paucisymptomatic infection. The impact of SARS-CoV-2 specific antibodies on SARS-CoV-2 susceptibility and COVID-19 severity after re-exposure remains still to be clearly determined.

Persistence

Depending on the studies, IgM level remains detectable in 80 to 97% of patients **up to 7 weeks** after the onset of symptoms. The concomitant presence of IgM and IgG for 7 weeks does not allow discrimination between patients during, at the end of, or after, infection.

A recent Iceland study demonstrates the persistence of Abs response up to 4 months. There are some data from China showing persistence up to 6 months.

Finally, regarding seroprotection, maybe cite the Addetia paper about the outbreak on a ship (attached). It is preliminary but encouraging and coincides with previous experience with endemic HCoV. (and NHP vaccine studies also...)

The longevity of the antibody response is still unknown, but it is known that antibodies to other coronaviruses wane over time (range: 12 – 52 weeks from the onset of symptoms) and homologous re-infections have been shown.

Preliminary results of a study performed in Sciensano in a cohort of mild-diseased health care workers is confirming

- the majority of subjects develop a vigorous humoral immune response with about 6% of the cases not developing detectable immune responses,
- the sustained presence of antibodies (IgG) in a majority of them after 16 weeks.

Recent studies have compared the antibody response in hospitalized COVID-19 patients and in mild or asymptomatic cases. Preliminary results (small cohorts) showed higher SARS-CoV-2-specific antibody responses in hospitalized patients compared to outpatients or asymptomatic individuals.

Some studies showed that humoral protection may be short-lived in patients with mild/no symptoms (this does not exclude the presence of T/B-cell memory response), for example neutralizing SARS-CoV-2 antibody levels decrease within the first 2 months after infection what is similar in anti-nucleocapsid antibodies of seasonal coronaviruses (53).

These findings highlight the pressing need for sensitive assays to increase detection of antibodies from pauci/a-symptomatic patients.

Level of antibodies for protection

The immune response is not always synonymous with protective immunization against new virus infections. This requires the production of sufficient levels of neutralizing antibodies over a long period of time.

Limited data suggest that neutralizing antibodies appear early following SARS-CoV-2 infection in humans and in a majority of patients. But the neutralizing antibody titer required for protection and the duration of neutralizing antibody production are not yet clearly defined.

Previous experience with SARS-CoV indicates that total IgG responses and neutralizing activity may persist at least 3 years in the majority of infected subjects (Cao NEJM 2007).

Studies are currently looking into the possibility for using different types of SARS-CoV-2 antibodies as a proxy for the neutralizing antibodies.

Preliminary results of a study performed by Sciensano using three different types of ELISA tests, targeting three different antigens (Nucleocapside, S1 and RBD) show that levels of S1- and RBD-specific antibodies correlate with neutralization antibodies when above a certain cut off. *Jun Wu et al* published an article indicating sustained humoral immunity in recovered patients who suffer from symptomatic COVID-19, suggesting prolonged immunity.

These results are encouraging and could support the implementation of serological testing as a proxy for protective immunity in exposed persons like health professionals. The sensitivity and the specificity of the tests will also have to be taken into account before recommending this indication.

6. New recommendations in international guidelines

6.1. ECDC

Last update August 10.

Sero-epidemiological studies can complement other surveillance approaches and allow for the monitoring of the proportion of the population that has previously been infected. These studies can be

carried out by testing cohorts of people in the community or in high-risk settings (e.g. households, healthcare settings) or through cross-sectional surveys (e.g. from blood banks). WHO Unity Study protocols are available for setting up such studies.

<https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-20200810.pdf>

6.2. WHO

Last update communication on immunity passport April 24

Some governments have suggested that the detection of antibodies to the SARS-CoV-2, could serve as the basis for an “immunity passport” or “risk-free certificate” that would enable individuals to travel or to return to work assuming that they are protected against re-infection. There is currently no evidence that people who have recovered from COVID-19 and have antibodies are protected from a second infection.

<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/serology-in-the-context-of-covid-19>

6.3. CDC

Last update August 24

The Food and Drug Administration has not authorized antibody testing to diagnose COVID-19, and the CDC does not currently recommend using antibody testing for stand-alone diagnosis of any infection. In certain situations, antibody tests may be used in conjunction with viral detection tests to support clinical assessment of persons who present late in their illnesses. In addition, if a person is suspected of having a post-infectious syndrome caused by COVID-19 (e.g., Multisystem Inflammatory Syndrome in Children; MIS-C), antibody tests may be used to determine prior infection.

Antibody tests for COVID-19 can play an important role in surveillance and epidemiologic studies, which can provide insights into the transmission dynamic of the virus among the general population. Unlike direct viral detection methods that can detect currently infected persons, antibody tests help determine whether the individual being tested was previously infected, even if that person never showed symptoms. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html>

In the same IDSA published recently: Information on the clinical performance and utility of SARS-CoV-2 serologic tests are rapidly emerging. Based on available evidence, detection of anti-SARS-CoV-2 antibodies may be useful for confirming the presence of current or past infection in 3 selected situations: 1) evaluation of patients with a high clinical suspicion for COVID-19 when molecular diagnostic testing is negative and at least two weeks have passed since symptom onset; 2) assessment of multisystem inflammatory syndrome in children; and 3) for conducting serosurveillance studies. The certainty of available evidence supporting the use of serology for either diagnosis or epidemiology was, however, graded as very low to moderate.

7. Self-tests

7.1. RATIONALE

Self-test (see definition in point 11) is useful when the result indicates to the patient that he/she has to seek medical attention. The self-test is easily available (without medical prescription in pharmacy – even on internet) and easily feasible by the patient (usually in a rapid test format).

Such a test does **not give a diagnostic**, it is a kind of screening tool that has always to be confirmed by additional tests (e.g.: pregnancy, HIV, ...). Self-test is an **orientation test** and to reach its goal, it has to detect the risk as early as possible.

All tests have intrinsic characteristics (sensitivity and specificity) and the accuracy is also depending on external factors like the moment of the test, the proper handling. Even if the test has good intrinsic characteristics, the result has to have an added value for the health of the patient.

Some of these rapid serological tests have been validated on serum, but there remains uncertainty on their use on capillary blood. Testing on finger prick blood is mostly less sensitive than on serum. A recent study performed by Sciensano comparing 5 different antibody-based rapid tests identified only one of them fulfilling the desired demands in terms of sensitivity and specificity. The searchers estimate the rapid test is at this moment best suited for screening purposes using finger prick blood in a large-based population setting, not for individual use.

The gaps in knowledge about immune response (See point 2) and the limitations of the serological tests (See point 11) are also applicable to Rapid serological tests.

As the purpose of a self-test is to inform the patient, within a short period of time, that he/she is probably at risk and that he/she should seek medical care, such a test could be relevant to the COVID-19 if:

- It can detect rapidly the infection
 - It can detect asymptomatic patients
 - A negative result indicates that the patient is not infected
- ➔ None of these objectives will be met because antibodies emerge usually between the 7 and 11th day after onset of symptoms in most patients while the strategy for tackling the COVID-19 is to have an early diagnose. The test to be performed is therefore a PCR as soon as symptoms appears or in high risk contacts.

As it is for all serological test, the self-test doesn't offer neither the following information:

That the patient has never met the virus?

The test is maybe done too early in the clinical presentation or too late as the persistence of antibody responses is still largely unknown. It is also possible that some antibody responses are too weak to be measured with current assays.

A positive result indicates no risk of contagiousness

No, even if a person has developed an immune response, he/she can still likely transmit virus if he/she is a carrier of the virus.

A positive result indicates a protection against a reinfection

We don't know yet if the presence of antibodies are protecting the person against reinfection.

In France, the test available in pharmacy since mid-July is a rapid antibody test, but it has to be performed in a pharmacy. Home testing is forbidden. The decision to make rapid serological tests available in pharmacies is also supported by the scattered medical services in some parts of the country.

Décrets, arrêtés, circulaires textes généraux ministère des solidarités et de la santé, Arrêté du 10 juillet 2020 prescrivant les mesures générales nécessaires pour faire face à l'épidémie de covid-19 dans les territoires sortis de l'état d'urgence sanitaire et dans ceux où il a été prorogé. It is a rapid test performed by a paramedical professionals, not really a self-test for the patient him/herself.

7.2. CONCLUSION OF SELF-TEST

The RAG agrees on the importance of the involvement of patients in management of their health and therefore the use of self-test when appropriate.

The RAG supports the position of the Association of Pharmacists Belgium (APB) when describing the scope of the Self-test: "Self-tests do not make a diagnosis, but for certain well-defined target groups they make it possible to determine the need for a medical consultation".

The RAG therefore assesses that the use of self-tests is not appropriate for COVID-19 because antibodies are not produced sufficiently early for adequate case management and interruption of the chains of transmission and all the limitations given here above. In Belgium, the medical offer is dense almost everywhere.

A member of the RAG is nevertheless mentioning that the availability of such a test could attract the public into a public health important topic by allowing asymptomatic seronegative person to do repetitive

serological testing in order to detect seroconversions. It is maybe more affordable options for certain type of organisations (ex.: sportclub, ...) also taking into account with possible shortage for PCR.

8. Place of serological tests

On the basis of current knowledge, serology alone does **not** allow to define whether a person:

- is infected with SARS-CoV-2 within the first week after symptom onset,
- has developed an asymptomatic/paucisymptomatic (sometimes undetectable threshold) infection,
- is protected against reinfection and if so, for how long,
- is no longer contagious,
- is in the course of infection or in the post-infection period.

Serology is used to determine whether a person has triggered an immune response to the virus.

Serological tests are therefore not recommended as first-line test for the early diagnosis of COVID-19 infection in the first week after the onset of symptoms.

9. Indications of serological tests

In blue the additional indications discussed by the RAG.

9.1. DIAGNOSTIC FRAMEWORK

According to the opinion of the RAG of 20/04, the place of serology for diagnostic purposes concerns:

- Most diagnostic difficulties emerge when patients are admitted in the second week of infection due to a lower sensitivity of the PCR. For hospitalized patients with a suggestive clinical picture and in case of divergence between PCR and CT Scan, by paired sera since the (5-)7th day of illness and a second one at least 7-10 days later (for both IgM and IgG). Serologic testing can also support diagnosis of acute COVID-19 illness for persons who present late (9–14 days after illness), only one a serum on 9–14 days after illness onset².
- For ambulatory patients with a suggestive and prolonged clinical picture but a negative PCR **or** who would not have benefited from PCR within 7 days after the onset of symptoms (Also by rapid test by first line care if tests validated on capillary blood).
- For differential diagnosis of atypical clinical presentation or to help support a diagnosis when patients present with late complications of COVID-19 illness.

9.2. CASE AND CONTACT MANAGEMENT

- Serological testing can be part of recognition of occupational diseases in the context of occupational medicine (but should not be charged to the clinical biology budget), but limited. It can be used for retrospective diagnostic confirmation taking into account the limitations : it is not possible to distinguish between the most probable source of exposition; private or professional exposition, antibodies can be not present in a certain number of HCW contracted the virus professionally or vanish by the moment of testing.
- Considering that some serological tests don't show very high specificity, in a low prevalence situation (as it is in Belgium), false positives cannot be neglected (with 8% prevalence and if 98% sensibility/specificity, false positive is reaching 20%).
- For high risk contacts of a patient with proven COVID-19, a positive serology test in combination with a negative first PCR might be **not** an indication to stop earlier the quarantine of this person. In addition, if the high risk contact is an household of the case, it is not excluded that the contact will carry the virus, and therefore transmit it, even if having an immunity because he/she is exposed to the case during his/her contagious period. The simultaneous use of the serology is therefore **not** recommended in such a situation.

² During the first two weeks following symptom onset the sensitivity for all serology tests, regardless of the platform and immunoglobulin detected, is inadequate to avoid a large number of false negative results. CDC.

9.3. THERAPEUTIC FRAMEWORK

- Serological tests could be useful in the monitoring of plasma from convalescent donors in a context of anti-COVID-19 therapy.

9.4. HEALTHCARE WORKERS / CAREGIVERS

Healthcare workers/caregivers are at the forefront of the response to COVID-19 and as such, have a risk of exposure to suspect cases but are also in contact with vulnerable individuals. The intensity of the crisis also exposes them to a situational risk related to long working hours, psychological distress, fatigue, burnout, ...

Protecting the healthcare workers /caregivers from these two risks is based above all on the following elements:

- Putting in place the specific prevention and protection measures necessary to maximize safety at work (e.g. personal protective equipment, hygiene, incident reporting, self-assessment, ...)
- Detecting any suspected cases

Testing of health care workers is indicated in the following situations:

- Any health care professional/caregiver at the onset of symptoms (extended case definition), by PCR
- Identification of failures in the application of protective measures and grouped cases (local risk management), by PCR and/or serology
- Description of the risk of exposure of health professionals (Public health monitoring, studies in a local risk management approach), by PCR and/or serology

In the case of clusters, a local testing strategy will be developed in consultation with the infectious disease prevention and control services of the health authorities. This is mainly carried out by a PCR test and is aimed at managing the event. In systematic screening like in nursing home, a serology can help in interpreting a PCR positivity with high Ct-values/low viral load surely in case of testing/screening of asymptomatic persons.

If the preliminary results of the study performed in Sciensano establish a correlation between level of antibodies and protection against reinfection, serology could be used to define the level of protection of the health professionals.

9.5. OTHER PROFESSIONAL CATEGORIES

Under no circumstances could a positive serology be used by the employer to decide on a subsequent shift in activity since it remains uncertainties whether the antibodies are protective.

Furthermore, it should be kept in mind that with a prevalence <8% in the general population, the positive predictive value of a serological test with good sensitivity/specificity is limited (+/- 80% if sensitivity/specificity > 98%). There would therefore be 20% false positives.

9.6. EPIDEMIOLOGICAL FRAMEWORK

Serological tests can be used to conduct epidemiological studies, the results of which will help in crisis management by describing herd immunity, both at the general population level and in certain risk groups.

These studies should be repeated at regular intervals to detect a change in herd immunity.

Sciensano has gathered information on the different initiatives underway, planned or future and submitted a surveillance plan that is covering for example the following aspects:

1. Level and duration of immunity in the general population
2. Level and duration of immunity in specific groups, according to demographic characteristics, ...
3. Level and duration of immunity among persons of different clinical severity
4. Existence of Immunity in Asymptomatic Patients
5. Probability of protection against reinfection

6. Evolution of viral load and the appearance of antibodies

These studies may be carried out by ELISA or rapid tests. Rapid capillary blood or saliva tests would be very useful for studies or surveillance in the general population, especially in primary care settings. The latter two tests have yet to be validated.

A list of ongoing, planned or proposed studies by different groups of researchers is available.

9.7. OTHER

- Use of serology should be evaluated in vaccine clinical trials and in measuring immunity when vaccination of the population will be launched. While taking into account the possible evolution of the knowledge, the place of serological tests will be evaluated when revising the testing strategy in case of an out-of-stock situation for molecular tests.

10. Annexes

10.1. TYPES OF SEROLOGICAL TESTS

More and more serological tests are available on the market, both commercial and in house.

10.1.1. ELISA

Sensitivity and specificity for the tests can be checked via <https://finddx.shinyapps.io/COVID19DxData/>.

To have the most accurate data, it is necessary to know which assays exactly.

10.1.2. Rapid diagnostic tests (RDTs)

When prevalence is high, Antibody-based RDT can be used to reduce the burden on the laboratory system. They can help to triage possible cases in a later phase of disease. However, in a low prevalence situation the PPV of these Ab RDT is too low to be clinically meaningful.

Limitations of rapid test:

- Only qualitative test.
- The time of positivity depends of the type of test and the type of antibodies (IgM can be present 1-3 days before IgG).
- Most of the rapid tests detects both IgM and IgG together
- Not yet validated on blood samples taken by capillary puncture.
- Interpretation of rapid-test results can be difficult if at the limit of detection.
- A positive result should be confirmed by an ELISA test (only screening purpose) and a PCR if IgM only are detected.

Belgium has some of these tests validated and there are large differences in quality between the tests. The FAMHP has also been informed of a number of frauds and this also includes a test marked with an IVD-EC. The tests used will have to be validated and carried out in a medical laboratory.

10.2. DEFINITIONS

Automated tests (ELISA) = qualitative or semi-quantitative test (titration) of the production of antibodies, only in a medical laboratory, on blood samples taken by venous blood sampling.

Rapid diagnostic test (usually immunochromatographic) = defined by EU as a qualitative or semi-quantitative in vitro-diagnostic medical devices, used singly or in a small series, which involve non-automated procedures and have been designed to give a fast result. These tests can be performed by patients, in medical care settings as point-of-care tests for rapid diagnostic orientation or in the context of survey. There are based on various types of parameters like antibodies, antigens, hormone, ... on blood, serum, urine, nasopharyngeal secretions, ...

The **self-test** is a biological examination designed to carry out a diagnosis at home thanks to rapid diagnostic test. There are self-tests for pregnancy, HIV, ...

11. Contributions

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