ADVANCING PHARMACY WORLDWIDE

FIP HEALTH ADVISORY

COVID-19:
Evaluation of diagnostic testing methods and devices

INTERNATIONAL PHARMACEUTICAL FEDERATION

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COVID-19: EVALUATION OF DIAGNOSTIC TESTING METHODS AND DEVICES

FIP will update this interim guidance as more information becomes available.

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Purpose of this document

The COVID-19 pandemic has created suffering for the whole world and an urgent need for diagnostic testing of patients and populations. In response, a multitude of COVID-19 tests are available to use from many different manufacturers and organisations. The SARS-CoV-2 testing working group, assembled by the leadership of FIP, has created this counselling information guide to assist practising pharmacists around the world in talking to patients who have questions concerning SARS-CoV-2 tests.

The summary of the charts and introduction material provide pharmacists with an understanding of the tests available. This information may be useful to everyone, whether they provide point-of-care tests or not.

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Background documents and acknowledgements

The two common types of tests available, diagnostic of current infection (i.e., antigenic/virological/molecular) and indicative of past infection (i.e., serological/antibody), are discussed in this document. The tables and information are based on the latest available resources (see references section) and information and charts generously provided by the American Society of Health-System Pharmacists (ASHP). The original version of ASHP COVID-19 resources can be found at the ASHP website and in the testing document.[1,2]

A link to tests that have received an Emergency Use Authorization (EUA) from the United States Food and Drug Administration (FDA) can be found here.[3]

A link to the authorised products from Health Canada can be found here.[4]

A link to the authorised products from the Australian Government Department of Health can be found here.[5]

Additionally, a similar summary of tests commonly used in France can be found here [6] and in Brazil here.

SARS-CoV-2: Basics of testing

Understanding antigenic tests

Antigenic tests detect one of the proteins of the SARS-CoV-2 virus (generally the nucleocapsid protein NP) from a nasopharyngeal or nasal sample and, like the detection of the viral genome by RT-PCR, would allow a diagnosis of early-stage SARS-CoV-2 infection. It should be noted that the efficiency of the detection of viral antigens seems to correlate with the viral load itself, decreasing with the number of days after onset of symptoms. Therefore, just as has been proposed for RT-PCR on saliva samples, it is proposed to consider antigenic test results only during the seven days following the onset of symptoms.

Compared with the reference test (RT-PCR on nasopharyngeal swab), the objective of antigenic tests is to accelerate and facilitate the performance of the test and its reporting of results, in particular to reduce the risk of viral transmission. Indeed, the antigenic tests carried out on nasopharyngeal or nasal samples can be rapid tests (15 to 30 minutes), easy to use and to interpret (unlike COVID-19 serological tests because of the presence or absence of several isotypes). The principle is generally based on immunochromatography with a reading which can be either manual or automated. Tests are mostly rapid unit tests (i.e., a rapid diagnostic test [RDT]) carried out by clinical laboratories and point-of-care tests (POCTs) by other operators. However, it should be noted that there are no data in the literature relating to the use of antigenic tests in asymptomatic patients in the context of contact-tracing or screening.

Mechanisms of antigenic tests

Most antigenic tests (Ag-tests) for COVID-19 use a "sandwich" immunodetection method employing a simple-to-use lateral flow test format commonly employed for HIV, malaria and influenza testing. Ag-tests are usually comprised of a plastic cassette with sample and buffer wells, a nitrocellulose matrix strip, with a test line with bound antibody specific for conjugated target antigen-antibody complexes and a control line with bound antibody specific for conjugated-antibody. In the case of SARS-CoV-2 Ag-tests, the target analyte is often the virus's nucleocapsid protein, preferred because of its relative abundance. Typically, all materials that are required to perform the test, including sample collection materials, are provided in the commercial kit, with the exception of a timer.

After collecting the appropriate specimen and applying it to the test strip, the operator can read the results within 10 to 30 minutes with or without the aid of a reader instrument. The use of a reader standardises interpretation of test results, reducing variance in assay interpretation by different operators, but requires ancillary equipment. Most of the currently manufactured tests require nasal or nasopharyngeal swab samples, but companies are carrying out studies to assess the performance of their tests using alternative sample types such as saliva, oral fluid and sample collection systems to potentially expand options for use and to facilitate safe and efficient testing. Generally, the ease-of-use and rapid turnaround time of Ag-tests offers the potential to expand access to testing and decrease delays in diagnosis by shifting to decentralised testing of patients with early symptoms. The trade-off for simplicity of operation of Ag-tests is a decrease in sensitivity compared with RT-PCR tests. Very few of the SARS-CoV-2 Ag-tests have undergone stringent regulatory review.

Place of antigenic tests

According to the World Health Organization (WHO), antigenic tests for SARS-CoV-2 with a sensitivity ≥ 80% and a specificity ≥ 97% compared with the reference RT-PCR test, can be used to diagnose a SARS-CoV-2 infection when RT-PCRs are not available or the clinical utility of screening would be compromised by over-lengthy delays in obtaining results.[23] Ag-testing is not recommended in asymptomatic persons unless they are confirmed contacts of someone who has already tested positive. Furthermore, to avoid false positives, the WHO does not recommend the use of rapid antigenic tests. Thus, antigenic tests are not recommended as a monitoring tool in conditions of low virus circulation. In this case, the RT-PCR test remains the reference test. To optimise performance, the WHO states that Ag-testing should be conducted by trained operators in strict accordance with manufacturers' instructions and within five to seven days following the onset of symptoms.

Situations where the WHO states that SARS- CoV-2 Ag-RDTs should not be used (based on currently available information)

Do not use SARS-CoV-2 Ag-RDTs in the following situations:	Explanation
In individuals without symptoms unless the person is a contact of a confirmed case	Pre-test probability (the likelihood, before testing, that the patient has the disease based on epidemiology, case contact, clinical findings) is low.
Where there are zero or only sporadic cases	Ag-tests are not recommended for routine surveillance purposes or case management in this circumstance. Positive test results would likely be false positives. Molecular testing is preferred.
Appropriate biosafety and infection prevention and control measures are lacking	To safeguard health workers, respiratory sample collection for any test from patients with suspected COVID-19 requires that operators wear gloves, gown, mask and face shield or goggles.[24-26]
Management of the patient does not change based on the result of the test	If test-positive and test-negative patients will be treated the same way because of unknown or low positive (PPV) and/or negative predictive value (NPV), then there is no benefit to be gained from testing.
For airport or border screening at points of entry	Prevalence of COVID-19 will be highly variable among travellers, and it is therefore not possible to determine PPV or NPV of test results. Positive and negative tests would require confirmatory testing to increase PPV and NPV for decision making.
In screening prior to blood donation	A positive Ag-test result would not necessarily correlate with presence of SARS-CoV-2 in the blood. Asymptomatic blood donors do not meet the definition of a suspect case.[27]

Many factors may affect the performance of antigen-detecting tests. Consequently, findings in clinical settings may be variable. The following should be taken into account:

- Patient factors, such as the time from illness onset and immune status
- Sample type (upper or lower respiratory tract), quality and processing, including storage conditions and dilution in virus transport medium
- Viral factors, including the concentration and duration of viral antigen shedding and structural variation in the target antigen, cross reactivity with other viruses
- Specific protein target, as some antigens are produced in higher concentrations than others, e.g., nucleocapsid versus spike proteins
- Product design or quality issues including:
    - Insufficient antibody quantity or affinity for the target antigen(s)
    - Poor packaging and exposure to heat and humidity during improper transport and/or storage, which can degrade antibodies in the test
    - Unclear or incorrect instructions that can affect test performance

- Inadequate training or competency of the test operator, which may lead to error in preparing the Ag-test, performing the test or interpreting the result, with erroneous conclusions

Implementation considerations of antigenic tests

Although Ag-tests may be considerably easier to perform than RT-PCR tests, they still require that supplier-recommended procedures be strictly followed with due attention paid to documentation, execution of time-dependent or volume-dependent steps, storage conditions and shelf-life, and equipment and stock management. All test operators must be trained in sample collection, relevant biosafety, performance of the test and interpretation and reporting of results, as well as in waste management. Quality control measures also need to be put in place.

Use of instrumented detection systems demands additional training requirements (instrument use, calibration as required, service requirements, operating conditions) and sufficient infrastructure, such as a reliable source of electricity.

Sample collection is one of the most critical factors affecting performance of Ag-tests. Instructions for use should be carefully followed, and any staff collecting samples should be trained in the methodology.

Each of these tests has a specifically indicated method for sample processing after collection. Instructions should be followed precisely, and no alternative reagents used (e.g., water or other liquid instead of dilution/mixing buffer).

Biosafety requirements for operators must be in place: personal protective equipment, biohazard waste bags and good ventilation are essential.

Understanding serological tests

Serological (antibody) tests should never be used to diagnose a current SARS-CoV-2 infection. They are used as an indicator for past infection and, at this time, researchers do not know if the presence of antibodies means that you are immune to COVID-19 in the future. The majority of serological tests are based on the detection of antibodies to highly immunogenic SARS-CoV-2 proteins, specifically the external protein spike (S) or the S1 subunit and the fraction thereof targeting the receptor-binding domain and the internal nucleoprotein (N). The serological kits are divided into rapid diagnostic immunochromatographic unit tests (also called LIFA [lateral flow immunoassays]) whose response is qualitative (positive or negative) and automated immunometric tests (ELISA, CLIA), the results of which are expressed qualitatively or semi-quantitatively depending on the reactivity index of the sample tested and with respect to a threshold value. These automated tests allow a large number of samples to be processed. Tests such as luciferase immunoprecipitation assay (LIPS), S-Flow assay, microarray, Luminex or the pseudo neutralisation or neutralisation tests are used in clinical research.

In biological practice, ELISA type diagnostic tests and rapid immunochromatographic tests are medical biology examinations. Some rapid tests can become point-of-care tests (POCTs or “doctor’s tests”), performed by healthcare professionals outside the clinical (or medical) laboratory. Although the COVID-19 doctor’s test meets the same specifications (sensitivity, specificity) as the other serological tests for the detection of SARS-CoV-2 antibodies, they cannot replace medical laboratory tests. While POCTs are most often carried out on capillary whole blood, the evaluation of their performance is carried out on plasma or serum of hospitalised patients. Note that as part of the tests’ CE marking, manufacturers are required to assess the performance of their tests on unmanipulated whole blood samples. In addition, they are most often performed in non-symptomatic or pauci-symptomatic people, which can have an impact on the kinetics of the antibodies.

In a recent (July 2020) systematic review of meta-analyses covering 40 bibliographic references analysing the intrinsic performance of serological tests, 10% of these

tests were performed in people followed in the outpatient setting. Two of these studies included POCTs and highlighted the heterogeneity of the tests.

As the number of testing kits increases in availability, caution in the quality of interpretation of POCT results is highly recommended. The interpretation of the serological profile of patients cannot be independent of the context in which these tests are performed, and diagnostic approach, symptoms and epidemiological situation should be considered.

Mechanisms of diagnostic tests and interpretation of serological results

An antibody is a protein produced by the body's immune system when it detects harmful substances, including viruses. Antibodies against protein N are detected earlier than antibodies against protein S. The latter is well correlated with the presence of neutralising antibodies.[7] Currently available kits are directed at identifying only one of these antigens (N or S protein).

Precise interpretation of a test's results depends on the prevalence of the infection being tested within a given population. This determines the positive predictive value (PPV), which is the probability that subjects with positive screening tests truly have the disease.[8] Since the PPV of serological tests is dependent on the prevalence of COVID-19 in the population studied, it is not relevant to perform systematic serological tests when the prevalence is weak. Simulations carried out using the minimum threshold values of sensitivity and specificity (90% and 98%, respectively) illustrate the predictive values according to prevalence assumptions.[9] In people who have been infected with COVID-19, PPV increases.

Antibodies, also known as immunoglobulins (Ig), are produced by the human immune system in response to a variety of stimuli, including viruses, as a part of adaptive immunity. IgM and IgG play specific roles in identifying different timelines of disease exposure and progression with recent roles in the SARS-CoV-2 disease. IgM antibodies are the first antibodies produced and can indicate an active or recent infection. Although the most common, IgG antibodies develop later following infection, and generally do not appear until approximately seven to 10 days after infection. The production kinetics of anti-SARS-CoV-2 antibodies have been essentially documented by the detection of IgG and IgM. However, it has been shown in a few studies that the detection of IgA is contemporaneous with that of IgM, on average five days after the onset of clinical signs (three to six days), with a seroconversion rate of 90% to 100% from day 15 to day 21.[10]

Detection of IgM and IgG is observed between day 5 and day 14 after the onset of clinical signs, with a median delay of five to 12 days for IgM and 14 days for IgG.[7, 10-16] Specifically for the SARS-CoV-2 infection, IgMs are detected during the first four weeks after the onset of infection, while IgG levels increase starting from week 2 after the onset of clinical signs.[17]

The kinetics of the humoral response vary depending on several factors and high titres (measuring the number of antibodies within a person's blood) of circulating antibodies are usually reached more quickly in severe cases of COVID-19. In a retrospective study conducted among 802 people in the Wuhan region of China, 21% of those who were asymptomatic had sequential SARS-CoV-2 RT-PCR (reverse transcription-polymerase chain reaction) and serum samples taken from either the upper or lower airways. Results showed that a positivity rate for IgG-type antibodies was higher in the cohort of symptomatic people than in the asymptomatic cohort.[17] However, a high antibody titre is not always associated with the elimination of the virus and is independently associated with clinical severity on multivariate analysis.[10, 11, 16]

The parallel detection of viral RNA (a molecular method like PCR used to quantify copies of small part of SARS-CoV-2, which is a RNA virus) and antibodies improves the sensitivity of the diagnosis of COVID-19 infection beyond the first week of evolution.[10, 16]

In pauci-symptomatic people taken as part of an investigation of grouped cases, approximately two weeks after signs suggestive of COVID-19, the seropositivity rate was on average 32% (27–37%) depending on the tests used.[7]

Does the presence of antibodies confer immunity to SARS-CoV-2?

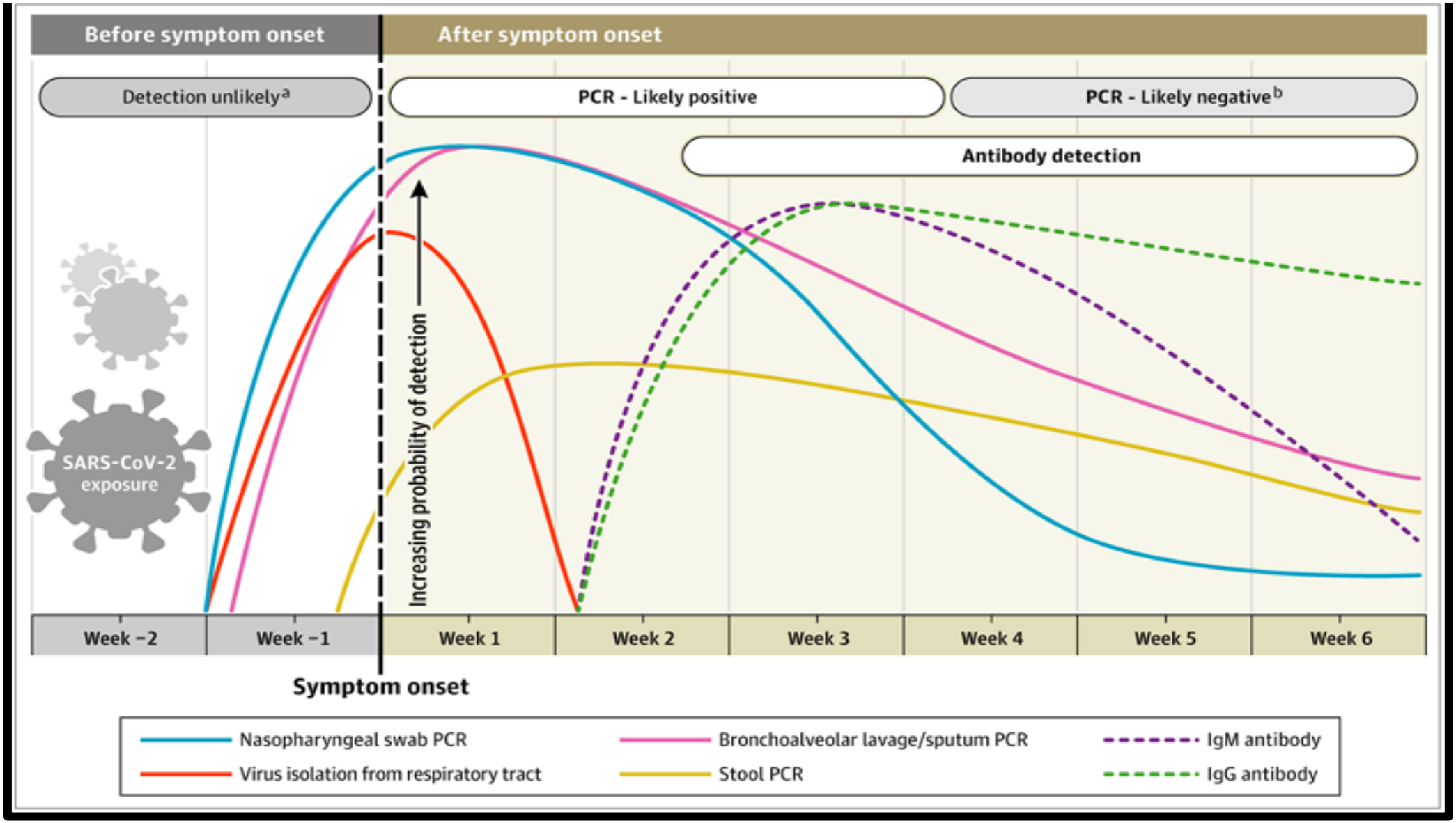
It is currently premature, given the lack of understanding of this new virus, to determine the level of protection (duration of immunity, titre of neutralising antibodies to ensure protection against (re)-infection with COVID-19). Several studies indicate that the antibodies detected in people who have recovered from COVID-19 are neutralising and appear seven to 15 days after the onset of clinical signs.[7, 14, 18]

The titre of neutralising antibodies does not correlate with the duration of the disease, however there are more neutralising antibodies found in the titres of patients aged 50 years and over. The neutralising response also varies depending on the symptoms, with a lower rate of neutralisation in pauci-symptomatic persons within 15 days after the onset of symptoms but comparable after one month.[7, 19, 20] In hospital staff with mild forms of SARS-CoV-2 infection, neutralising antibodies were detected in 79%, 92% and 98% of the samples taken, respectively, 13–20, 21–27 and 28–41 days after the onset of symptoms.

The search for virological markers was carried out with 390 regular blood donors at the beginning of April 2020 in part of Lombardy, Italy, at a time when SARS-CoV-2 was actively circulating. (There was a suspension of blood donation collection in this region from 20 February to 27 April, at the time of the study.) The clinical investigation indicated that 20 of the donors demonstrated clinical signs suggestive of COVID-19 within 30 days of collection of nasopharyngeal and serum samples. Of the remaining donors, 23% (n=91) had neutralising antibodies, with more than 65% having a low titre. SARS-CoV-2 RNA was detected in 4.3% of donors (n=20) of whom three also had neutralising antibodies.[21] These data underline that the study of the correlation between the presence of neutralising antibodies and the level of protection that these antibodies can confer against infection by SARS-CoV-2 should be continued.

The humoral response appears to be limited in time with the drop in the titre of IgG-type antibodies and neutralising antibodies observed two to three months after infection. This is observed more specifically in people who have presented asymptomatic or pauci-symptomatic forms (Figure 1 below).[20, 22]

Figure. 1. Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset



Estimated time intervals and rates of viral detection as presented by several published reports. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction.

Reference: Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 2020 May 6. doi: 10.1001/jama.2020.8259

Summary and key points

To summarise:

- The detection of SARS-CoV-2 by RT-PCR is a powerful method for the diagnosis of COVID-19 in the acute phase within the first two weeks after the onset of clinical signs.
- The use of antigenic tests for the diagnosis of SARS-CoV-2 infection is more restrictive and depends on the organisational choices of the national health authorities, which pharmacists must refer to and respect.
- The presence of anti-SARS CoV-2 IgG-type antibodies allows for retrospective diagnosis of SARS-CoV-2 infection beyond the first two to three weeks after the onset of clinical signs and symptoms.
- The presence of anti-SARS-CoV-2 IgM-type antibodies is a marker of recent infection.
- During the acute phase of COVID-19, serum antibodies can be detected by automated immunometric technique or by a rapid immunochromatographic test. Neutralising antibodies are detected later by neutralisation technique (not performed in routine laboratory practice) are detected later in the asymptomatic or pauci-symptomatic forms.
- The titre of IgM-type antibodies is also lower in asymptomatic or pauci-symptomatic persons compared with those presenting with a more severe illness.
- The antibodies detected in medical biology practice reflect exposure to the virus. The correlation with their protective activity remains to be established.
- During SARS-CoV-2 infection, a neutralising-type immune response has been detected but seems to decrease two to three months after infection, particularly in people who are asymptomatic or pauci-symptomatic.
- The surge of testing kits for SARS-CoV-2 has pressed local and national regulatory agencies to scrupulously evaluate these tests to ensure standards of quality, and to validate the appropriate tests for public use. Follow the guidelines of these regulatory agencies regarding the approved testing devices to use.
- With the rapid emergence of new technologies to test for SARS-CoV-2 and the dynamic nature of the virus, it is important to scrutinise and remain vigilant when assessing such technologies for use outside of hospitals.
- There is a need for further scientific investigation into the dynamics and quality of the humoral response, while the cell-mediated immune response remains to be documented.

Conveying testing information to the public

General information about SARS-CoV-2 PCR (or virological) and antigenic testing

Not everyone needs to be tested. However, people with someone with known exposure, living with a person who has tested positive, or people with recent travel to highly affected areas may need to be tested.

Patients with viral symptoms may also need to be tested. These include:

- Temperature greater than 37.5–38°C/99.4–100.4°F
- Non-productive cough
- Runny nose
- Body aches
- Muscle and/or joint pain
- Headache
- Nausea, vomiting and diarrhoea
- Extreme tiredness, fatigue
- Darkening colour of the toes
- Loss of appetite
- Loss of the ability to smell or taste

The diagnosis of SARS-CoV-2 infection is based on the detection of the viral genome or the virus itself on samples from the respiratory tract (virologic and antigenic tests), or on the detection of anti-SARS-CoV-2 antibodies in certain specific indications from local health authorities, particularly in the context of the post-infection diagnosis.

General testing information

- Tests are not perfect and can occasionally give the wrong result.
- The timing of the test can affect the value of the results.
- Tests run too early or late may give a false negative result.

Counselling on SARS-CoV-2 testing

What to tell a patient who has tested positive

- Having this virus means that you can be contagious.
- You need to practise social distancing, wear a mask, wash your hands frequently and comply with all recommendations put in place by local health authorities.
- Since you have the virus, you should stay at home and avoid close contact with others if possible until your healthcare provider tells you that you are well enough to resume your normal daily activities.
- If you share accommodations with others, here are some considerations to take to protect the spread of the virus to them.
- Remain separated from others as much as possible
- Wear a mask when you are in the same room as another person
- Use a separate bathroom, if available
- Wipe down the surfaces of communal areas of the house as much as possible
- Do not share a room with people who are at risk of severe disease (elderly, immunocompromised, those with heart, lung or kidney conditions, or those with diabetes)
- Having a positive test does not tell us when or how you were infected.
- You should monitor your health.
- Take your temperature twice every day
- Contact your healthcare provider if you notice increased difficulty in breathing
- Contact your healthcare provider if you or those around you notice that you appear to be confused or that you have increasing difficulty with cognitive focusing.
- Expect to be tired.
- Recovery will take time. You will have good days and bad days, that is expected. It is important to rest whenever you are able.

- The virus is detected one to two days before the onset of clinical signs and can persist for up to eight days in moderate forms of COVID-19 or even two to four weeks after the onset of clinical signs in the more severe forms.
- It has been possible to observe a positive response to virologic tests a few days or even several weeks after a negative response, sometimes accompanied by reappearance of clinical respiratory signs when these people had been considered as cured.

What to tell a patient who has tested negative

- A negative test does not mean that you don't have the virus; it means that the test was unable to identify the virus.
- You should still practise social distancing, wear a mask, wash your hands frequently, and comply with all recommendations put in place by local health authorities.
- It can take up to one week for test results to show positive.
- Virologic tests go negative three to four weeks after onset of symptoms.
- If you develop signs of COVID-19, you may need to be retested.

Counselling on SARS-CoV-2 antibody testing

What to tell a patient who has tested positive

- A positive antibody test does indicate that that the virus has been eliminated from the body.
- We do not yet have evidence that antibodies provide full protection against this virus. Continue to practise all necessary safety considerations (social distancing, hand washing, mask wearing, covering your mouth and nose when coughing or sneezing) and comply with all recommendations put in place by local health authorities.
- A positive antibody test does indicate that you have been previously infected by the virus (there can be interference from other coronaviruses).
- The presence of anti-SARS-CoV-2 IgM-type antibodies is a marker of recent infection.
- Do not hesitate to make a confirmation of the rapid test by a serological examination in a medicine laboratory directly or indirectly with a prescription.
- Long term effects from COVID may impact your breathing or your heart. It is important to tell all medical providers that you have been infected and be monitored.

What to tell a patient who has tested negative

- Antibodies don't appear for at least a week after symptoms appear, and two weeks after SARS-CoV-2 virus test shows positive. It is possible that you have the virus but have not yet developed antibodies.
- There have been reports of patients who had antibodies at one time, testing negative at some time later.
- Where a patient with symptoms of COVID-19 has been in contact with a person ill with COVID-19, the absence of antibodies does not allow us to say that the patient has not been in contact with the SARS-CoV-2 virus (due to the lack of sensitivity of the serological kit used).
- If the test only or also detects IgM antibodies, results naturally turn negative six to seven weeks after symptom onset, and eight to nine weeks after a positive PCR test.

Important notes for the pharmacist

Considerations when talking to patients

- Remember to ask about antipyretics use before taking a patient's temperature.
    - acetaminophen/paracetamol/aspirin/non-steroidal anti-inflammatory agents
- Non-productive ("dry") cough is a difficult concept for some patients; be prepared to explain what you mean.
    - Influenza cough starts out productive; COVID-19 cough is rarely productive.
- Patients can be contagious for up to 72 hours before developing any symptoms; remember always to protect yourself.
- Carefully read the technical leaflets of the POCTs that you propose to patients, and more particularly which antibodies (IgM, IgG, both, or total Ig) they detect, and do not hesitate to inquire with the supplier in case of doubt.

Key points to tell patients regarding specific tests

RT-PCR test

- The RT-PCR test is the gold standard test for patients with current COVID-19. RT-PCR testing will only detect patients who are currently infected with COVID-19. This test does not show if the patient has had COVID-19 in the past or has current immunity to COVID-19. This test might cause a patient to get a false negative result if the patient is tested at an early stage of infection.[28, 29]

LAMP test

The LAMP test can be used as a point-of-care test due to quick test results and decreased equipment costs. However, the patient needs to know that point of care testing has more variable sensitivity than RT-PCR testing and therefore has a higher risk of false positives and false negatives.[28, 30, 31]

CRISPR test

- The CRISPR test represents a prime example of where mixed technologies can lead, combining high specificity with efficient and low-cost biosensors. It can also be run several times to decrease the chances of false negatives.[28, 29]

Antigenic tests

- Antigenic tests may provide faster results, but they also have lower sensitivity and specificity than molecular testing and therefore are prone to more false negatives.[28-30]

Table 1. Diagnosis of current infection (virological tests)

Fundamental features	Feature	Essential	Rationale
	Clinical sensitivity	>98% Sensitivity	Lower sensitivity makes test results clinically insignificant.
	Clinical specificity	>98% Specificity	Lower sensitivity makes test results clinically insignificant.
Test procedure			
	-	Must be self-contained	More complex testing should only be conducted in full laboratories with strict safety guidelines and testing procedures.
Operational characteristics			
	Quality control and standards	Must meet standard qualifications to be certified to perform testing on human samples	Tests must meet quality standards set by local and/or national health authorities to be able to test human specimens.
	Biohazard	Must not generate medical waste	-

Table 2. Diagnosis of current infection (antigenic tests)

Fundamental features	Feature	Essential	Rationale
	Clinical sensitivity	>80% Sensitivity	Lower sensitivity makes test results clinically insignificant.
	Clinical specificity	>97% Specificity	Lower sensitivity makes test results clinically insignificant.
Test procedure			
	-	Must be self-contained and include everything required to perform and quality control the test	More complex testing should only be conducted in full laboratories with strict safety guidelines and testing procedures.
Operational characteristics			
	Quality control and standards	Must meet standard qualifications to be certified to perform testing on human samples	Tests must meet quality standards set by local and/or national health authorities to be able to test human specimens.
	Biohazard	Must generate little medical waste	-

Table 3. Diagnosis of past infection (serological tests)

	Feature	Essential	Rationale
Fundamental features			
	Target population	People who need to know they have previously been exposed to SARS-CoV-2	People may have recovered from suspected or confirmed SARS-CoV-2 infection or they may have previously developed an asymptomatic infection. Positive test does not necessarily mean immunity has been conferred.
	Intended use	Detection of IgG antibodies to SARS- CoV-2 in venous or capillary blood	Assist screening for past SARS-CoV-2 infection. Not suitable for diagnosing active infections.
	Patient interaction	Limited	Fewer interactions between suspected cases and the people administering the test (testers should avoid repeated or prolonged contact with patients while performing the test).
	Affordability	-	Tests and related equipment should be affordable to for both end-users (test providers) and the target population (patients)
	Clinical sensitivity	> 98%	Ability to correctly identify patients who have had COVID-19 (true positive). Anything less provides unactionable information.
	Clinical specificity	> 98%	Ability to correctly identify patients who have never had COVID-19 (true negative). Anything less provides unactionable information.
	Ease of use/administration	At least one control per device lot number, better with one control per box or storage package	Tests and related equipment should be easy to use for any end-user. One control per lot number will allow for a standard of affordability. One control per box or storage package will allow to detect any kind of poor conservation.
	Deliverability to end-users	Should be easily accessible to those performing the tests	-
	Involved equipment	Only the basic materials provided to accomplish appropriate testing	Should involve limited steps and included equipment (i.e., no multiple reagents).
	Analytical specificity	No cross reactivity with other coronaviruses	An absence of interference and cross reactivity provides results specific for SARS-CoV-2.
	Usable on capillary blood	Fully validated on capillary blood	Venous blood and capillary blood differ markedly in their composition, with potential impact on the sensitivity and sensitivity of the test.
Test procedure			
	Need for operator to transfer a precise volume of sample or reagent	No	Limited steps prevent risk of human error and false results.

	Sample preparation	No more than 15 minutes. None or fully integrated	No need to process sample prior to performing the test.
	Regulatory status	CE marked/EUA approved CLIA waived in the US IVDR waived in EU	-
	Type of analysis/result output	Qualitative	Describes presence of antibodies in sample rather than amount.
	Involvement of outside facility/lab	Minimal involvement	In-house testing allows for prompt results and contact isolation if necessary.
	Time to results	Under 20 minutes (but note that some of the RT-PCR tests run for several hours)	Prompt results allows for quick implementation of contact isolation and treatment.
	Biosafety	No biosafety should be needed in addition to personal protective equipment (PPE)	Prevents excessive use of PPE.
	Ease of interpretation of result	Easy	Minimises user-to-user variability to ensure accurate results.
Operational characteristics			
	Training requirements	None	No need for time dedicated to training end-users.
	Operating conditions	5–30°C, 80% relative humidity	Range of working conditions to allow for more widespread use in different climates.
	Power requirements	None	To allow for use in times of power outages.
	Disposal requirements	None. Device and accessories should be disposed of in standard biological waste containers	-
	Reagent reconstitution	None. Reagents come ready to use	No need to prepare reagents prior to use.
	Volume of sample	POCT: single blood drop for fingerprick tests	Want the minimum required amount but enough to ensure sensitivity of sample processing.
	Need for calibration	None	Little to no calibration allows for more accurate and precise results as more tests are performed.

The following tables have been adapted from ASHP's COVID-19 Diagnostic Testing Chart and represent molecular tests most widely used in North America.

Table 4. Overview of molecular testing

Adapted from ASHP COVID-19 Diagnostic Testing Chart

COVID-19 test type	Molecular testing — major examples			Antigenic tests
	RT-PCR	LAMP	CRISPR	
Term definition	Reverse transcriptase polymerase chain reaction (RT-PCR).	Loop-mediated isothermal amplification (LAMP).	Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR).	Rapid test for viral proteins or antigens of COVID-19.
What is the test identifying?	SARS-CoV-2 viral RNA presence[28]	SARS-CoV-2 viral RNA presence[28]	SARS-CoV-2 viral RNA presence[28]	Presence of the nucleocapsid protein antigen[32]
When should the test be used? Detectable COVID-19 virus begins approximately 3 to 7 days after exposure and peaks with symptom onset.[22, 30]	Diagnosis of COVID-19 during the acute phase of infection Asymptomatic patients with COVID exposure Symptomatic patients[28]	For quicker diagnosis of COVID-19 during the acute phase of infection Asymptomatic patients with COVID exposure Symptomatic patients[28]	For quick diagnosis of COVID-19 during the acute phase of infection Asymptomatic patients with COVID exposure Symptomatic patients[28]	For quick diagnosis of COVID-19 during the acute phase of infection Asymptomatic patients with COVID exposure Symptomatic patients[28]
How is the test sample obtained from the patient?	Various upper respiratory specimens (nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, nasal mid-turbinate swab, saliva)[28, 32]	Various upper respiratory specimens (nasopharyngeal, oropharyngeal swab, anterior nasal swab, saliva)[28, 31]	Nasopharyngeal or oropharyngeal[32]	Nasopharyngeal or oropharyngeal[32]
Who can obtain the test sample?	All tests can be performed by a healthcare provider or trained operators Nasal mid-turbinate swab can be performed by a supervised patient Anterior nasal swab and saliva collection can be performed by a supervised or unsupervised patient[32]	All tests can be performed by a healthcare provider or trained operators Nasal mid-turbinate swab can be performed by a supervised patient Anterior nasal swab and saliva collection can be performed by a supervised or unsupervised patient[32]	Tests can be performed by a healthcare provider or trained operators[32]	Tests can be performed by a healthcare provider or trained operators*[32]
What does a negative test mean?	Does not rule out COVID-19 due to chance of false negatives[28]	Does not rule out COVID-19 due to chance of false negatives[28, 31]	Does not rule out COVID-19 due to chance of false negatives[28]	Does not rule out COVID-19 due to chance of false negatives[32]

Does the test need to be repeated?	This test should be repeated in a patient with a negative test and high clinical suspicion of COVID-19 as this test will not detect if the patient is at an early infection stage and the patient could still develop symptoms in the future. The test should not be repeated after a positive COVID-19 test as the test will still be positive after the resolution of symptoms due to the detection of small viral RNA particles.[28, 29]	This test should be repeated in a patient with a negative test and high clinical suspicion of COVID-19 with a RT-PCR test. The test should not be repeated after a positive COVID-19 test as the test will still be positive after the resolution of symptoms due to the detection of small viral RNA particles.[28, 31]	This test should be repeated in a patient with a negative test and high clinical suspicion of COVID-19 as this test will not detect if the patient is at an early infection stage and the patient could still develop symptoms in the future. The test should not be repeated after a positive COVID-19 test as the test will still be positive after the resolution of symptoms due to the detection of small viral RNA particles.[28, 29]	The test should be repeated if both the positive and negative controls fail during the testing process. Additionally, a negative antigenic test may require confirmatory testing with a molecular based diagnostic test prior to making treatment or prevention decisions.[32]
*Allowing supervised or unsupervised patients to obtain samples may decrease the desirable clinical sensitivity and specificity of the test, previously mentioned in Table 1				

Table 5. Overview of diagnostic test specimen collection method

Adapted from ASHP COVID-19 Diagnostic Testing Chart

Specimen collection method: Nasopharyngeal swab[33, 34]		
Description of collection method	**Who may collect the specimen**	**PPE required**
1) Have the patient remove their mask 2) Ask the patient to blow their nose into a tissue 3) Remove swab from packaging 4) Tilt the patient's head back slightly 5) Ask the patient to close eyes to lessen mild discomfort 6) Gently insert swab along nasal septum and parallel to the palate (not upwards) to nasopharynx until resistance it felt 7) Gently rub and roll swab and leave the swab in place for several seconds 8) Then, slowly remove the swab while rotating the swab several times 9) Ask the patient to put their mask back on 10) Open the collection tube and insert the swab into the tube 11) Break the swab at the groove and discard the remaining swab 12) Close the labelled collection tube, wipe with surface-disinfectant wipe and place it in a biohazard bag	Healthcare provider	▪ Protective gown ▪ Non-sterile gloves ▪ Protective mask with a rating of N95 or high ▪ Face shield or goggles

Specimen collection method: Oropharyngeal swab[31, 32, 34]		
Description of collection method	**Who may collect the specimen**	**PPE required**
1) Have the patient remove their mask 2) Remove swab from packaging 3) Swab the posterior pharynx, tonsils, or other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab 4) Ask the patient to put their mask back on 5) Open the collection tube and insert the swab into the tube 6) Break the swab at the groove and discard the remaining swab 7) Close the labelled collection tube, wipe with surface-disinfectant wipe and place it in a biohazard bag	Healthcare provider	▪ Protective gown ▪ Non-sterile gloves ▪ Protective mask with a rating of N95 or higher ▪ Face shield or goggles
Specimen collection method: Nasal mid-turbinate swab[34]		
Description of collection method	**Who may collect the specimen**	**PPE required**
1) Ask the patient to remove their mask 2) Remove swab from packaging 3) Tilt the patient's head back 70 degrees 4) Direct mid-turbinate swab straight back along nostril floor until stopper hits the nostril. 5) Rotate the swab several times (10–15 seconds), then slowly remove from the nostril 6) Repeat in the other nostril using same swab 7) Ask the patient to put their mask back on 8) Open the collection tube and insert the swab into the tube 9) Close the labelled collection tube, wipe with surface-disinfectant wipe and place it in a biohazard bag	Healthcare provider or supervised patient	If the pharmacist is collecting the specimen then: ▪ Protective gown ▪ Non-sterile gloves ▪ Protective mask with a rating of N95 or higher ▪ Face shield or goggles If the patient is obtaining the specimen and the pharmacist is collecting it then: ▪ Distance from the patient (ideally 1.5–2m) ▪ Gloves and mask
Specimen collection method: Anterior nasal swab[34]		
Description of collection method	**Who may collect the specimen**	**PPE required**
1) Have the patient remove their mask 2) Remove the direct nasal swab from packaging 3) Carefully insert the swab into the nostril exhibiting the most visible drainage or the nostril that is most congested if drainage is not visible	Healthcare provider, supervised patient, or unsupervised patient	If the pharmacist is collecting the specimen then: ▪ Protective gown ▪ Non-sterile gloves ▪ Protective mask with a rating of N95 or higher

4) Using gentle rotation, push the swab until resistance is met at the level of the turbinate (less than one inch or less than 25mm into the nostril) 5) Rotate the swab several times against the nasal wall and leave in place 10 to 15 seconds then slowly remove from the nostril 6) Using the same swab, repeat the sample collection in the other nostril 7) Ask the patient to put their mask back on 8) Open the collection tube and insert the swab into the tube, then immediately run the POCT test		▪ Face shield or goggles If the patient is obtaining the specimen and the pharmacist is collecting it then: ▪ Distance from the patient (ideally 1.5–2m) ▪ Gloves and mask
Specimen collection method: Saliva sample[34-36]		
Description of collection method	**Who may collect the specimen**	**PPE required**
1) Ask the patient not to drink any water for 10 minutes prior to the test and to avoid food, other liquids, or teeth-brushing for 30 minutes prior to the test. 2) Have the patient remove their mask 3) Hand the specimen cup to the patient 4) Ask the patient to allow the saliva to pool in their mouth for a few seconds without swallowing and then lean forward and let it drip (do not spit or cough) into the specimen cup repeatedly until roughly one third of the cup is full or up to 2ml full of liquid (excluding bubbles) 5) Ask the patient to securely close the specimen cup before handing it back to the healthcare worker 6) Ask the patient to put their mask back on 7) Wipe the specimen container with surface-disinfectant wipe and place it in a biohazard bag	Healthcare provider, supervised patient, or unsupervised patient	If the pharmacist is collecting the specimen then: ▪ Protective gown ▪ Non-sterile gloves ▪ Protective mask with a rating of N95 or higher ▪ Face shield or goggles If the patient is obtaining the specimen and the pharmacist is collecting it then: ▪ Distance from the patient (ideally 1.5–2m) ▪ Gloves and mask

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Validity

This document was prepared based on commonly accepted evidence as of November 2020.

Disclaimer

The information contained in this document is rapidly evolving because of ongoing research and is subject to the professional judgement and interpretation of the practitioner. This document is based on the available evidence and the recommendations of reputable organisations such as the World Health Organization, the United States and the European Centres for Disease Control and Prevention, among others, as cited at the time of publishing. The available knowledge about COVID-19 is rapidly changing and recommendations may change accordingly. Although FIP will strive to keep these guidelines up to date, we recommend consulting the websites of these organisations and any newly available evidence for the most recent updates.

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