Guidance note on SARS-CoV-2 testing

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Background
Testing is a critical component of epidemic response, as it allows public health authorities to identify both individual cases and emerging hotspots of COVID-19, and to rapidly implement effective epidemic control measures such as isolation and treatment and contact tracing around confirmed cases to slow and stop the spread of the virus. National Societies, as auxiliaries to their public health and emergency response authorities, may be asked to support government testing strategies to identify and rapidly isolate cases. This guidance provides explanations of the types and reliability of different test types and strategies. It is meant to inform effective decision-making by National Societies involved in or considering support to COVID-19 testing.
Test sensitivity and specificity

No test is 100% accurate. Some tests may result in a positive person testing negative (false negative) or a negative person testing positive (false positive). Both false negatives and false positives can have a significant impact on individual health and on the impact of epidemic response measures. The quality of tests is often discussed in terms of sensitivity and specificity.

- Sensitivity is the proportion of people who are actually infected with the virus who test positive, i.e. a true positive. A test that is 100% sensitive means that everyone who has the virus will receive a positive test result. A sensitive test is used to exclude a disease, as it rarely misclassifies someone who does have the disease as “healthy”.
- Specificity is the proportion of people who are not actually infected with the virus who test negative, i.e. a true negative. A highly specific test means that healthy people are correctly identified as healthy, and there are no false positives. A specific test is used to rule in a disease, as it rarely misclassifies someone who doesn’t have the virus as “infected”.

Types of COVID-19 tests

Nucleic acid amplification tests (NAAT): real-time reverse-transcription polymerase chain reaction (rRT-PCR) (also called “PCR”)

This test serves to identify viral genetic material (RNA) by searching biological samples for a pattern corresponding to the RNA sequence of the pathogen to be identified and its subsequent amplification to quantify it. It can be used both as a qualitative test (“positive or negative”) and as a quantitative test, indicating not just the presence of the virus, but also the viral load (i.e. “how much”). For COVID-19, samples are collected from the upper respiratory tract, usually with a swab that is rubbed on the oral-pharyngeal mucosa or nose (in ICU or other health facilities, samples can also be collected from the lower respiratory tract). It is processed in the laboratory by qualified personnel with specific equipment. Usually, results take a few hours (although depending on the volume of tests managed by the lab and the severity of the case, it can take several days).

The test is capable of detecting the presence of viruses, both in symptomatic and asymptomatic people, and was the only accepted test for the diagnosis of COVID-19 until late September 2020, when antigen Rapid Diagnostic Tests became available, as announced by the Access to COVID-19 Tools (ACT) Accelerator (see below). The estimated sensitivity of PCR tests is between 71 – 98%, and can vary based on the timing of the test (when it was conducted compared to when a person contracted SARS-CoV-2, which is also why subsequent tests are suggested in clinical settings). False-positive results are rare, but they do occur, particularly as a result of contamination or human error while taking or handling samples. False-negative results are more common in presymptomatic patients, with the test’s sensitivity increasing as the patient becomes symptomatic. In a review of seven studies (1,330 respiratory samples) using RT-PCR, there was a 100% false-negative rate four days before symptom onset, decreasing to 38% false negative on the day of symptom onset, and 20% three days after symptom onset. False negatives increased again as the disease progressed, rising to 66% 17 days after symptom onset. In any case, in periods longer than 10 days, and always in the absence of symptoms, the result of the PCR, in relation to the infective capacity of the person, varies; and could determine different clinical and epidemiological consequences.

The PCR test remains the most specific and sensitive test currently available for COVID-19.
Rapid diagnostic tests (RDTs) based on antigen detection
These tests detect the presence of viral proteins (antigens) expressed by the COVID-19 virus in a sample from the respiratory tract of an infected person. Antigen tests only work if there is enough virus, and therefore viral proteins, in the sample to bind to specific antibodies contained in the test. These tests often look like a paper strip, and a detectable sign (usually a coloured band) appears to indicate the presence of antigens. The antigen(s) is detected only if the virus is replicating, so these tests may identify acute or early infection. However, many factors could interfere with results, resulting in a vast spectrum of sensitivity and specificity, depending on the specific test used.5

That said, as a result of research and development efforts made by the Access to COVID-19 Tools (ACT) Accelerator, launched by the WHO, a set of agreements were announced in September 2020 to make available high-quality and affordable (US$5/unit) COVID-19 Antigen Rapid Diagnostic Tests (Ag-RDTs) to Low and Middle Income Countries. These tests can be used at the point-of-care and provide results in 15-30 minutes, therefore enabling expansion of testing particularly in countries without extensive laboratory facilities or trained health workers to perform PCR tests.6

Ag-RDTs are less sensitive than nucleic acid tests, which means they are not as accurate at identifying people who are positive. However, they are generally very specific, which means they are very accurate at identifying people who are negative. To be useful, Ag-RDTs need to correctly identify significantly more cases than they miss (sensitivity ≥80%) and have very high specificity (≥97-100%). To optimize performance they should therefore be conducted in strict accordance with the manufacturer’s instructions and within the first 5-7 days following onset of symptoms. As well as supporting test-trace-isolate strategies, the tests can help identify or confirm new outbreaks, support outbreak investigations through screening; monitor disease trends; and potentially test asymptomatic contacts. As mentioned above, PCR testing remains the most specific and sensitive test for clinical diagnostic detection of SARS-CoV-2, but Ag-RDTs offer opportunities in settings where PCR testing is unavailable or prolonged turnaround times preclude clinical and public health utility.7

Rapid diagnostic tests based on host antibody detection
These are the most common RDT marketed for COVID-19. These tests detect the presence of antibodies in the blood (usually by pricking the person’s fingertip). There are two types of antibodies: IgM and IgG. IgM can be detected approximately seven days after the onset of symptoms, and they disappear roughly after 21 days; they may reflect an active phase of the infection. IgG appear around 14 days after the onset of symptoms, and they may remain for some time (for some diseases, for life) and they may confer immunity (protection against reinfections or decrease the severity or new clinical courses). While these tests are critical to support vaccines development and researches into attack rate in the population and fatality rate, for instance, at different geographical levels, they have very limited utility for clinical diagnosis as they cannot be used to inform any decision on the clinical outcome or even active surveillance (a negative test doesn’t mean a person is not infected, as that person can be infected and contagious, but antibodies are not present yet). Also, so far, there is no evidence that the presence of IgG confers protection or immunity against future reinfection with the virus that causes COVID-198.

Both antigen and antibodies rapid tests are qualitative; they can say whether or not there is the presence of either viral proteins or host antibodies, but they cannot provide any information about quantity. Antigen and antibody tests can also be done in the laboratory using more precise quantitative tests; however, the indications and their utility do not differ from those mentioned for rapid tests.
What does WHO recommend?

Prior to September 2020 the only test accepted by WHO to consider a person has been tested for COVID-19 is a person who has been screened for the virus with nucleic acid amplification tests (NAAT), such as rRT-PCR. However, in September 2020, the Access to COVID-19 Tools (ACT) Accelerator—a global health collaboration launched by WHO—announced agreements with Abbot and SD Biosensor to make available high quality antigen rapid diagnostic tests (Ag RDTs) to low and middle countries. It is important to emphasize that to date, only those two antigen RDT tests have been endorsed by WHO.

Based on current evidence, WHO recommends the use of immunodiagnostic tests like antibody tests only in research settings, but they shouldn't be used for any clinical decision-making or modification, both at individual and community level, of Public Health and Social Measures (PHSM).10

What if my MOH recommends something else?

Different Ministries of Health may have different policies regarding the types of tests, whom to test and how to use the results. Local health authorities must determine these standards and strategies and must decide whether or not they align with the international standards set by WHO and other regulatory institutions. Where appropriate, NS are encouraged to advocate for alignment with international normative standards based on scientific evidence and should try, to the extent of their ability, expertise and independence, to use such international guidelines in their interventions. In no case, we can recommend NS make their own decisions informed on testing strategies with no sound scientific evidence.
# How to interpret test results

<table>
<thead>
<tr>
<th>Test type</th>
<th>Interpretation of the result</th>
<th>Infectiousness</th>
<th>In the absence of country protocols, next steps to consider:</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT / PCR tests</td>
<td><strong>+</strong> The person who has tested positive is classified as a confirmed case of COVID-19, whether or not they have symptoms.</td>
<td>The infected person is likely able to transmit the virus, especially prior to and in early symptom development. If the person is in recovery, they may still test positive for the virus but be less likely to transmit SARS-CoV-2.</td>
<td>The person should be treated as if they are infectious, isolated to prevent transmission, and receive follow-up and medical care if needed. Contacts should be traced.</td>
</tr>
<tr>
<td></td>
<td><strong>-</strong> A negative RT-PCR test indicates that the virus is not found in the collected sample and that, therefore, it is not infected by SARS-CoV-2. False negatives are more common than false positives, but both are relatively rare. In most countries, determining that a person is “cured” of COVID-19 (after a positive PCR test) requires at least two negative PCR tests spaced by at least, 24 hours³.</td>
<td>A presymptomatic or symptomatic person who tests negative may still be able to transmit the virus. Someone who is recovering from COVID-19 tests negative is unable to transmit the virus.</td>
<td>A person with clinical symptoms of COVID-19 and a negative test result should be retested and treated as contagious until further results. A clinical evaluation must accompany all laboratory diagnosis; in case of doubt or inconsistency between laboratory and clinical tests, repeat the RT-PCR and look for other infections with similar symptoms.</td>
</tr>
<tr>
<td></td>
<td><strong>+</strong> The person who has tested positive probably has active SARS-CoV-2 infection, whether or not they have symptoms. False positives are common.</td>
<td>If a true positive, the person is infectious.</td>
<td>In situations where confirmatory testing with NAAT/PCR is not feasible, any indications that results may be incorrect should raise suspicions about validity. Examples would include patients who are test-positive but have a clinical syndrome not consistent with COVID-19, or patients with a positive test detected in a low-prevalence setting (where false positives are more likely).</td>
</tr>
<tr>
<td></td>
<td><strong>-</strong> The person is very unlikely to be currently infected with SARS-CoV-2. A negative test</td>
<td></td>
<td>The person should be considered negative, while remaining alert to indications of a false negative. A negative Ag-RDT result</td>
</tr>
<tr>
<td>WHO-Approved(^1) Antigen tests</td>
<td>-</td>
<td>result indicates that no viral material was detected in the sample. False negatives are rare.</td>
<td>If a true negative, this person is not infectious</td>
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</tr>
<tr>
<td>Official WHO guidance is pending</td>
<td>+</td>
<td>The person who has tested positive can be interpreted as experiencing or having experienced active SARS-CoV-2 infection, whether or not they have symptoms. However, results are unreliable, and at this time, immunity based on positive antigen testing is not well understood. Sensitivity and specificity of non-WHO approved antigen tests vary widely.</td>
<td>Unclear based on test results. The infected person, if genuinely positive, can transmit the virus.</td>
</tr>
<tr>
<td>Other Antigen tests</td>
<td>-</td>
<td>A negative test result indicates that no viral material was detected in the sample. Still, these tests, so far, do not have the minimum necessary indices of confidence, to guide any decision about clinical or public health measures (therefore, in addition, they are not usually found on the market and are practically not used).</td>
<td>Unclear based on test results. The person, if genuinely negative, cannot transmit the virus.</td>
</tr>
</tbody>
</table>

\(^1\) As of October 2020, the WHO-approved antigen tests are those developed by Abbott and SD Biosensor.
<table>
<thead>
<tr>
<th>Antibody tests</th>
<th>The person who has tested positive may have been previously infected or currently infected, depending on the results. However, tests are unreliable, and this may be a false positive.</th>
<th>Unclear based on test results. If IgM is detected without IgG, the person may be in the active phase of the disease, and contagious. If both IgM and IgG are detected, the person would be in an early recovery phase, and infectious. If IgG is detected without IgM, the person may be in the recovery phase, and not contagious. These interpretations are indicative, they must be consistent with the clinical evaluation, and in no case do they rule out the presence of the virus and therefore, contagiousness and the possibility of transmission.</th>
<th>Testing with PCR should be used to confirm whether someone has COVID, and to confirm that a COVID-19 patient has recovered and is no longer contagious.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>The person who has tested negative shows no evidence of past or present SARS-CoV-2 infection. However, tests are unreliable, and this may be a false negative.</td>
<td>Unclear based on test results. A negative antibody detection test could indicate that antibodies against SARS-CoV-2 were not detected at that time, but this does not indicate whether they are infected with the virus. The first antibodies (IgM) generally require at least a week after the onset of symptoms to be detected (and for IgGs more than two weeks).</td>
<td>Therefore, both in the latency and incubation period, as in the early phase of the disease, antibodies can NOT be detected, and this test will be negative, even though the person may be infected, ill and contagious (in addition to the possibility of a false negative). For this reason, a negative antibody test result does NOT yield any information about the infection and contagiousness status of the person tested.</td>
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</tbody>
</table>
Can we buy the tests locally using IFRC funding?
Tests and laboratory equipment fall under medical procurement and must be coordinated through Geneva. Geneva may identify local authorised suppliers of medical equipment. National Societies can also use tests provided directly by their Ministry of Health.

When are WHO-approved rapid diagnostic antigen tests most useful?
Ag-RDTs are not appropriate for all contexts or scenarios. See the [WHO guidance](https://www.who.int) for full details. WHO recommends that they can be appropriately used in the following scenarios:

<table>
<thead>
<tr>
<th>Scenario / Context</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbreaks in remote settings, institutions or semi-closed communities where PCR is not immediately available.</td>
<td>Multiple positive Ag-RDT results from multiple suspect cases within the same population strongly suggests that a COVID-19 outbreak is occurring in this population, and early action to control transmission should occur. Where possible, all samples giving positive Ag-RDT results (or at least a subset) should be transported to laboratories with NAAT/PCR capability for confirmatory testing.</td>
</tr>
<tr>
<td>In outbreaks in closed or semi-closed groups (e.g. schools, care-homes, ships, prisons, work places, dormitories) where an outbreak has already been lab-confirmed.</td>
<td>Once the presence of COVID-19 has been confirmed by NAAT/PCR, Ag-RDTs can be used to screen at-risk people and rapidly isolate and contact trace positive cases.</td>
</tr>
<tr>
<td>To monitor trends in disease incidence in communities or groups with community transmission, particularly among essential and healthcare workers during outbreaks.</td>
<td>Note that in communities with very low prevalence of COVID-19, Ag-RDTs will return many false positives and may not be useful.</td>
</tr>
<tr>
<td>For early detection and isolation of positive cases in health facilities, COVID-19 testing sites, care homes, prisons, schools, and for resting of healthcare workers and contacts of cases, in areas with widespread community transmission.</td>
<td>Ag-RDT use in these contexts, where many people can be expected to be infected, are a useful tool to quickly screen people into isolation to prevent further transmission.</td>
</tr>
</tbody>
</table>

WHO recommends that they should not be used in the following scenarios:

<table>
<thead>
<tr>
<th>Scenario / Context</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>In individuals without symptoms unless the person is a contact of a confirmed case</td>
<td>The chance that the person is positive is low.</td>
</tr>
<tr>
<td>Where there are zero or only sporadic cases</td>
<td>When used for routine surveillance or case management when prevalence is low, most positive test results would likely be false positives. Thus, this will create more work than it saves.</td>
</tr>
<tr>
<td>Appropriate biosafety and infection prevention and control measures (IPC) are lacking</td>
<td>Do not undertake any kind of testing that endangers staff or volunteers. Collecting respiratory samples requires the sample-taker to wear gloves, gown, mask, and faceshield or goggles.</td>
</tr>
</tbody>
</table>
### Testing strategies

#### Who should be tested?

**Targeting**

The Ministry of Health and local health authorities set the national or local testing strategy. The target population will vary depending on the type of test and the purpose of testing. Before September 2020, the only test approved and recommended by the WHO for case detection was RT-PCR. This test is generally applied to those with symptoms compatible with COVID-19 to confirm the clinical diagnosis and proceed to patient isolation and care. The COVID-19 diagnostic test (RT-PCR) can also be performed in people without symptoms in screening activities or control of contacts; and, more usually, in people who, for example, are going to undergo surgery or require hospital admission for another cause. Health authorities generally adopt these latter applications of the RT-PCR test to maintain control of the epidemic and, therefore, the resources needed to carry out the PCR tests may no longer be exclusively devoted to meeting the urgent need to confirm the diagnosis in symptomatic people.

**Test type selection**

In September 2020, agreements to make antigen rapid diagnostic tests available to low and middle income countries were announced by the Access to COVID-19 Tools (ACT) Accelerator, launched by the WHO. These are currently manufactured by two companies only and are faster and cheaper than laboratory-based tests. As such, they enable countries to increase the pace of testing, tracing and treating people for COVID-19 at the point of care particularly in areas with under-resourced health systems. Immunological tests for the detection of antibodies against SARS-CoV-2 on the other hand, are recommended only in the context of research or in the estimation of the extent of past infection within populations. However, testing in these contexts does not inform clinical decisions or support immediate epidemiological control actions. These tests, therefore, can be applied to entire populations, population samples or specific groups, within the framework of these research purposes or determination of past exposure in the target groups.

**Sampling strategies**

**Screen in positive cases (symptom- or exposure-based testing):** The standard strategy is to test individuals with symptoms or suspicions of COVID-19. This requires tests to have high sensitivity (i.e. the test correctly identifies people who are infected), such as PCR testing.

**Screen out negative cases:** This strategy is not currently used broadly for COVID-19. Rather than seeking to identify people who are infected, it seeks to identify people who are not infected, so they can go on with their work, daily lives, etc. This requires tests to have high specificity (i.e. the test correctly identifies those who are not infected), but not necessarily high sensitivity.

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**Management of the patient does not change based on the result of the test.**

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Impact of Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>For airport or border screening at points of entry</td>
<td>Prevalence among travellers is highly variable. The number of false positives may be very high and vary according to the population tested, making it impossible to make effective decisions based on the results.</td>
</tr>
<tr>
<td>In screening prior to blood donation</td>
<td>A positive RDT would not necessarily correlate with presence of viremia.</td>
</tr>
</tbody>
</table>
**Pooled testing:** Instead of testing each person’s sample individually, pooled testing joins samples from a small number of people (e.g. 5-20 people’s samples together). If the pooled sample tests negative, all the people who formed that pool can be deemed negative. If the pooled sample tests positive, samples are tested in smaller batches or individually to identify the positive sample. This allows existing lab capacity to be used more efficiently. This strategy has been successfully used in China, Uruguay, Rwanda and many universities among other locations, and modelling also suggests its potential benefits in communities with low prevalence. The same strategy has been used to sample untreated human waste-water for SARS-CoV-2 infection, and can detect a positive COVID-19 case in a single person in a population of 10,000.

**How can NS support government testing strategies?**

Local health authorities have the power and regulatory authority to determine whom to test, when and with what type of test(s). If these authorities require NS support for this activity, IFRC recommends a careful evaluation of the protocols to be implemented. If said protocols are not aligned with international standards, a constructive technical communication could be established to try to advocate for the adoption of those criteria with a higher scientific basis. National Societies are encouraged not to participate in testing activities and strategies that may involve making high-risk decisions regarding both clinical care and epidemiological surveillance decision-making, and of course, in the case of violation of rights or ethical principles of individuals or communities. Although the refusal to support government activities may indeed carry present and future operational risks, the reputational and visibility risks of being associated with erroneous practices of an uncertain result but with high risk and impact should be considered more relevant.

If the support requested for testing activities is part of the research (such as the case of seroprevalence studies with antibody tests), this support could be considered, always evaluating the associated risks; knowing that it is the health and investigating authorities that must lead the strategic process as well as those that must provide the tests. In this case, it must also be very carefully evaluated whether the use of resources (material and human) in these research activities meet a minimum criterion of efficiency and impact. They do not negatively affect other activities of more significant impact at the preventive level, primary care, etc. that may be more necessary at the specific moment of the epidemic and more aligned with the role and the particular appeal of the Movement.

The majority of National Societies do not have an existing laboratory capacity. It is not recommended to develop laboratory capacity for the specific purpose of responding to COVID-19. However, antigen rapid diagnostic tests recently announced by the WHO-led Access to COVID-19 Tools (ACT) Accelerator, provide opportunities for countries that do not have extensive laboratory facilities or trained health workers to implement PCR tests. Appropriate testing strategies also require non-technical support at the community level, which some National Societies may be well placed to provide. This includes social mobilisation for testing campaigns, risk communication and community engagement to inform the public about testing, support to run testing facilities or support testing campaigns in target populations, and support for sample collection (where technical capacity exists) or other RDT roll-out using WHO-approved antigen tests.
My NS has hospitals/blood banks/laboratories and the competence to do advanced blood testing for other diseases. Can we start testing for COVID-19?

If the NS laboratory has the necessary equipment and professionals have the required training, RT-PCR tests could be performed for COVID-19. Some aspects that we consider relevant should be evaluated, such as:

- What material and human resources can be assigned to such activity.
- How will routine laboratory activities be affected by the referral of resources for the new intervention?
- How to ensure the sustainability of the activity while the implementation is imperative or the support request is maintained (it must be considered that poor planning with a potential abrupt interruption of the activity can entail very high clinical and reputational risks)

Does IFRC have a global COVID-19 testing strategy?

No, because the testing strategy of the NS should be aligned with the country’s MOH. On the global level, IFRC follows the normative guidance of the WHO.

Our MOH is asking us to buy them a PCR machine. What should we take into consideration?

Funding to expand testing is available through WHO country plans and other institutional donors and in-country mechanisms. There is consistent feedback that more significant community support is needed to carry out testing strategies at the population level effectively. Before exploring whether to support the MOH to purchase equipment which can be funded through other partners, National Societies should first consider whether all community-level elements of testing—where National Societies have a specific and unique presence and added value—are covered in affected communities, including:

- Social mobilisation for mass testing,
- Risk communication, community engagement and accountability (RC/CEA) to ensure people are informed about testing strategies and can provide feedback to health authorities,
- Support for testing facilities, including crowd control, mobile testing sites, etc. (run by MOH or other clinical partners),
- Support to people who test positive and require home isolation,
- Contact tracing around all positive cases, and support for contacts who are in quarantine in a facility or in their own homes.

Testing is one part of a multi-pronged strategy to slow and stop transmission, which also includes isolation and treatment of the sick, contact tracing, and other public health measures. Investing in testing without these activities – many of which can be vastly improved through the involvement of National Society branches and volunteers – significantly limits the impact of testing strategies.

Testing for research purposes

Since immunological antibody tests are not recommended to guide clinical decisions or real-time epidemiological surveillance, these tests are not eligible for humanitarian funding. For this reason, their
use and purchase cannot be funded through IFRC’s global COVID-19 appeal. It is recommended that the actions of the Red Cross Red Crescent humanitarian response be aligned with a criterion of efficiency and maximisation of impact, in line with the principles of optimal use of fields of action where they have greater knowledge and experience. Without underestimating the importance of research, we consider that it is the national authorities, regulatory institutions and reference research centres that can optimally undertake this activity, creating the required research protocols, monitoring their development, sharing the results and adopting both clinical and public health decisions that are considered pertinent.

In any case, if the decision is taken to carry out research work by its own choice and with its own funds, it is highly recommended to follow the standard steps for this type of activity: creation of a research committee, creation of an ethical committee, validation of research protocols, etc.

What resources are available to support testing?

Trainings and guidance
- Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance, 19 March 2020
- Protocol for real-time (RT)-PCR assays for the detection of SARS-CoV-2 for two RdRp targets (IP2 and IP4)
- Antigen detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim guidance, 11 September 2020

Operational considerations
- Swab storage (PCR)
  - The swabs can be stored dry or in a small amount of NaCl solution; if necessary, this should be clarified with the laboratory beforehand
- Sample storage (heat and transportation) (PCR)
  - Quick PCR examination is essential, preferably on the same day if possible.

References
