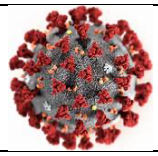
# National COVID-19 Science Task Force (NCS-TF)



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## An update on SARS-CoV-2 detection tests


## Summary of request/problem

Biological tests occupy an important place in the management of the COVID-19 pandemics. Two main categories of commonly used biological tests can be distinguished. First, the so-called virus detection tests, which aim at identifying individuals that are actively infected by SARS-CoV-2. Second, serological tests, which determine whether someone has antibodies against the virus, whether following a natural infection or a vaccination. The present paper describes how SARS-CoV-2 was identified and isolated and provides an update on virus detection tests currently utilized in Switzerland, as they are a cornerstone of our national strategy to monitor and contain the epidemics.


## Main text

1. How was SARS-CoV-2 identified?

SARS-CoV-2 was originally isolated by exposing cells in culture to samples harvested from the respiratory tract of individuals presenting with symptoms of what became known as COVID-19, in Wuhan, China, at the end of 2019. This is a standard approach when diagnostic tests for agents classically responsible for a given illness all yield negative results and one suspects that an unknown virus may be causing the disease. A virus, if placed in the presence of cells endowed with all the necessary properties, will enter and multiply in these targets. More virus will be produced and released in the culture supernatant, and in the case of coronaviruses the cells will ultimately undergo a so-called “cytopathic effect”, meaning that they will die, a phenomenon easily observed with a microscope. The supernatant of these cells can nevertheless be used to infect new cells, and if through several rounds of this process one repetitively observes the cytopathic effect one can conclude that a virus has been isolated. This is what was done with samples from COVID-19 patients first in China and later in many other countries to obtain SARS-CoV-2 isolates (Caly et al., 2020; Pohl et al., 2020; Zhu et al., 2020). These were then characterized by a variety of methods and assays to decipher the sequence of the viral genome (by sequencing techniques), to identify viral proteins (by using antibodies), and even to visualize the virus (by electron microscopy). Once this characterization was performed with samples from COVID-19 patients, it became clear that the virus had similarities with coronaviruses previously detected in bats and that it belonged to the species “severe acute respiratory syndrome-related coronavirus”. The International Committee for the Taxonomy of Viruses accordingly named this novel coronavirus “SARS-CoV-2”.

2. A brief description of the virus.

The genome of SARS-CoV-2, which bears the genetic information introduced by the virus in the infected cell, is made of a chain of nucleic acids called RNA. Some 30'000 bases, either A, U, G or C, constitute this genomic RNA, aligned like beads on a string in a sequence that is highly specific to the virus, that is, not found in our genome and differing markedly even from that of other coronaviruses. Once inside target cells, SARS-CoV-2 replicates its RNA to many copies, which serve either to trigger the production of viral proteins or to be incorporated in new viruses as these are released to the outside. Interestingly, SARS-CoV-2 is endowed with a proofreading capacity that minimizes the number of mutations introduced into new copies of its genome. Accordingly, the virus has not undergone major changes since it first emerged in the human population. This explains that its biological properties and virulence have not fundamentally evolved. This also is a good omen for the efficacy of future vaccines (Rausch et al., 2020). Still, minimal differences in the 30,000 bases sequence can be observed over time, enough to trace the virus as its spreads from individual to individual (information on the sequence of viral isolates circulating in Switzerland and elsewhere in the world can be obtained at *Nextstrain*[1]). In the virion, that is, in the vehicle that propagates the infection from cell to cell and from one person to the next, the viral genomic RNA is packaged together with several different viral proteins, each present in many copies, some inside the particle some on its surface. Virus detection tests were designed to detect either the viral RNA or the viral proteins through techniques that are distinct and proper to each one of these two entities.

3. SARS-CoV-2 detection tests

   a. Tests targeting the viral genome

The SARS-CoV-2 RNA can be detected by approaches relying on the amplification of its *in vitro* synthesized DNA copy by either RT-PCR (reverse-transcription polymerase chain reaction) or by a related technology called RT-LAMP (reverse-transcription loop-mediated isothermal amplification). The uniqueness of the SARS-CoV-2 genome sequence is key to the specificity of these assays.

As of today, RT-PCR is by far the SARS-CoV-2 detection test most commonly used in Switzerland. It relies on the use of two to three sets of probes that hybridize to the viral genome in a highly specific fashion, and serve as primers for its amplification. As a result, RT-PCR is both highly specific (because of the precision of the probes used) and highly sensitive (because it amplifies the signal present in the sample). The SARS-CoV-2 RT-PCR assay was developed as soon as the first viral genomes were sequenced, which allowed for the design of sets of PCR primers targeting the virus. Thousands of pre-pandemic samples have been tested in Swiss laboratories since the start of the pandemics, and results with currently used tests were uniformly negative indicating that neither other coronaviruses nor other respiratory viruses endemic in the human population give rise to falsely positive RT-PCR results. Furthermore, last June when the incidence of the infection in Switzerland was low, the percentage of positive tests went on some days all the way down to 0.2%, indicating that the rate of false positive is way below this value since one must assume that a large fraction of these positive results, obtained in individuals tested because they presented symptoms compatible with COVID-19, were true positives[2]. RT-PCR is a quantitative assay, each cycle of amplification (performed with a so-called thermocycler) doubling the number of DNA copies made from the initial reverse transcription product of the viral RNA. A fluorescent readout allows a quantification of these DNA copies. The CT (cycle threshold) value is defined as the number of cycles required for the fluorescent signal to cross a threshold above background levels. CT values are thus inversely proportional to the amount of viral RNA present in the sample (i.e. the lower the CT value the greater the amount of SARS-CoV-2 RNA in the sample). Commonly used machines perform 40 cycles of amplification, and CT values of 37 and below are generally considered as unequivocally positive,

[1] https://nextstrain.org/sars-cov-2/

[2] https://covid-19-schweiz.bagapps.ch/fr-3.html

but even higher values raise a strong suspicion of infection owing to the extreme specificity of the technique.
RT-LAMP has not been subjected to the same degree of streamlining as RT-PCR. Its reagents are less standardized, and as a result it is less widely available and so far has not been used extensively. Nevertheless, RT-LAMP could in principle offer a lower-cost, fast and portable method to detect SARS-CoV-2 infection, as it does not require a thermocycler. Accordingly, this approach has been advertised as a form of "rapid diagnostic test". Preliminary evaluations in a Swiss reference laboratory suggest that it can reach a degree of specificity close to that of RT-PCR, but that its sensitivity is lower, meaning that it is less efficient at detecting small amounts of virus.

b. Test targeting viral proteins

Tests aimed at detecting viral proteins have recently become available, which are commonly referred to as "viral antigen tests" and are the most common type of rapid diagnostic tests. They do not rely on any amplification procedure, but on the triggering of a colorimetric reaction following the recognition of an abundant virion protein constituent (typically the so-called N protein) by a specific antibody. While an early generation of such tests, released during the first wave of the pandemic, was of poor quality, ongoing evaluations of recently released antigen tests indicate high levels of specificity, but a sensitivity that is significantly lower than that of RT-PCR, as expected from the absence of any amplification procedure[3].

4. Source of sample

Evidence accumulated so far indicates that nasopharyngeal swabs remain the most reliable sample source to detect SARS-CoV-2, whether through RNA- or protein-targeting tests. While oropharyngeal swabs may represent an acceptable alternative, for instance in children where nasopharyngeal swabbing may be challenging, saliva seems to contain generally less virus (Nacher et al., 2020), which results in decreasing the sensitivity of the overall procedure irrespectively of the virus detection test performed downstream.

5. Is there anything like a truly "rapid" test?

The antigen-based and LAMP methodologies have been advertised as rapid tests, because the detection reaction itself takes no more than 15 to 30 min for the former, around 45 min for the latter, and requires no or only technically simple equipment. However, the need for a nasopharyngeal swab as the sample source implies limitations in both time and scale, as a well-trained "swabber" can generally harvest samples from no more than 10-12 individuals per hour. Furthermore, interpreting the results of these tests requires a professional eye, and registration for reporting to the cantonal authorities is mandatory. Accordingly, while antigen tests are predicted to play an important role as complements of RT-PCR tests and as first choice in some indications, they cannot be envisioned for large-scale rapid screenings, for instance of the crowd at the door of a sport arena. In the future, point-of-care RT-PCR tests sensitive enough to allow the use of saliva as a sample source might bring us closer to having a truly rapid testing method.

6. Infected versus contagious

An infected individual is one that harbors the virus; a contagious individual is one that can pass it to others. Immediately after SARS-CoV-2 enters the body, viral levels present even at the point of entry are below the detection limit of all currently available tests. As the virus starts replicating in airway cells, it progressively emerges from this eclipse period, and the amounts of virions present in the respiratory system steadily increase to reach levels that peak more or less when symptoms first appear, on average 3-5 days post-inoculation, stay high for 3 to 4 days and then slowly decrease, at least in uncomplicated cases (Cevik et al., 2020). Because of its high sensitivity, RT-PCR commonly

[3] https://www.finddx.org/covid-19/

starts detecting the virus 2-3 days before the occurrence of symptoms, and remains positive around five to seven days after these abate, although low levels of virus can be detected for longer periods in rare individuals. In contrast, less sensitive assays such as antigen-based tests, because they require higher levels of virus to trigger a signal, turn positive later (only shortly before or concomitant with symptoms onset) and return to negative faster (some 4-5 days after symptoms onset). Therefore, antigen-based tests cannot ensure that someone is not infected. However, they may suffice to detect a vast majority of individuals who are contagious, as contagiousness is generally proportional to viral load. Still, this assumption has two major caveats. First, individuals with symptoms enhancing viral spread, such as cough, will be prone to transmit the virus to others even at low viral loads. Second, if virus levels are in their ascending phase, a negative antigen test could become positive if sampling is repeated 1-2 days later.

For specific information on the relative performance of particular SARS-CoV-2 detection tests, the reader is invited to consult https://www.finddx.org/covid-19/, where results of tests evaluation by reference laboratories are constantly updated.Information on the sequence of viral isolates circulating in Switzerland and elsewhere in the world can be obtained at https://nextstrain.org/sars-cov-2/.

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