# Diagnostic testing for SARS-CoV-2

Interim guidance
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World Health Organization

## Introduction

This document provides interim guidance to laboratories and other stakeholders involved in diagnostics for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It covers the main considerations for specimen collection, nucleic acid amplification testing (NAAT), antigen (Ag), antibody (Ab) detection and quality assurance. This document will be updated as new information becomes available. Feedback can be sent to WHElab@who.int.

**Changes from the previous version**

The title of this interim guidance has changed from "Laboratory testing for COVID-19 in suspected human cases" to "Diagnostic testing for SARS-CoV-2". Additional relevant background information and a clinical diagnostic algorithm has been added to the document. Furthermore, the guidance has been updated with new findings from the literature and best practices.

**Relevant WHO documents**

WHO has developed interim guidance and technical briefs to assist policy-makers and laboratories on testing for SARS-CoV-2. These documents cover laboratory testing strategy [1], laboratory assessment tool [2], laboratory biosafety [3], advice on the use of point-of-care immunodiagnostic tests [4], antigen detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5], guidance for the investigations of clusters [6], public health surveillance [7] and operational considerations for surveillance using GISRS [8]. In addition, early investigation protocols [9] can be used by countries to implement epidemiological studies and enhance understanding of transmission patterns, disease severity and prevalence, clinical features and risk factors of SARS-CoV-2 infection.

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**Background on SARS-CoV-2**

WHO was first alerted to a cluster of pneumonia of unknown etiology in Wuhan, People's Republic of China on 31 December 2019. The virus was initially tentatively named 2019 novel coronavirus (2019-nCoV).

Subsequently the International Committee of Taxonomy of Viruses (ICTV) named the virus SARS-CoV-2 [10]. COVID-19 is the name of the illness caused by SARS-CoV-2.

SARS-CoV-2 is classified within the genus *Betacoronavirus* (subgenus *Sarbecovirus*) of the family *Coronaviridae* [11]. It is an enveloped, positive sense, single-stranded ribonucleic acid (RNA) virus with a 30-kb genome [10]. The virus has an RNA proofreading mechanism keeping the mutation rate relatively low. The genome encodes for non-structural proteins (some of these are essential in forming the replicase transcriptase complex), four structural proteins (spike (S), envelope (E), membrane (M), nucleocapsid (N)) and putative accessory proteins [12-14]. The virus binds to an angiotensin-converting enzyme 2 (ACE2) receptor for cell entry [15-17].

SARS-CoV-2 is the seventh coronavirus identified that is known to infect humans (HCoV). Four of these viruses, HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43, are endemic, seasonal and tend to cause mild respiratory disease. The other two viruses are the more virulent zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus type 1 (SARS-CoV-1). SARS-CoV-2 is most genetically similar to SARS-CoV-1, and both of these viruses belong to the subgenus *Sarbecovirus* within the genus *Betacoronavirus* [11]. However, SARS-CoV-1 is currently not known to circulate in the human population.

The clinical presentation of SARS-CoV-2 infection can range from asymptomatic infection to severe disease [18-27]. Mortality rates differ per country [28]. Early laboratory diagnosis of a SARS-CoV-2 infection can aid clinical management and outbreak control. Diagnostic testing can involve detecting the virus itself (viral RNA or antigen) or detecting the human immune response to infection (antibodies or other biomarkers).

While our understanding of SARS-CoV-2 has rapidly expanded, there are still many outstanding questions that need to be addressed. WHO encourages research and the sharing of results that may contribute toward an improved characterization of SARS-CoV-2 [29, 30].

**Background information on SARS-CoV-2 RNA detection**

Standard confirmation of acute SARS-CoV-2 infections is based on the detection of unique viral sequences by nucleic acid amplification tests (NAATs), such as real-time reverse-transcription polymerase chain reaction (rRT-PCR). The assays' targets include regions on the E, RdRP, N and S genes.

Once an individual has been infected by the virus, the mean time it takes to develop symptoms (incubation period) is 5-6 days, with a range of between 1 and 14 days following exposure [31-35]. The virus may be detectable in the upper respiratory tract (URT) 1-3 days before the onset of symptoms. The concentration of SARS-CoV-2 in the URT is highest around the time of symptom onset, after which it gradually declines [36-42]. Some studies report higher viral loads in the severely ill compared with patients with mild disease, while other studies do not report such differences [36, 43-49]. The presence of viral RNA in the lower respiratory tract (LRT), and for a subset of individuals in the faeces, increases during the second week of illness [38]. In some patients the viral RNA may only be detectable for several days, while in other patients it can be detected for several weeks, possibly months [44, 50-60]. Prolonged presence of viral RNA does not necessarily signify prolonged infectiousness. Several studies describe the correlation between reduced infectiousness and i) increased number of days that have elapsed since symptom onset and resolution,  ii) decrease in viral load in respiratory secretions [37, 61-64] and iii) an increase in neutralizing antibodies [37, 61]. More information can be found on this in Criteria to release COVID-19 patients from isolation [65].

Respiratory secretions may be quite variable in composition, and the adequacy of sampling efforts may also vary, which can occasionally result in false-negative PCR results [40, 42, 58, 66-74]. In patients for whom SARS-CoV-2 infection is strongly suspected and URT swabs are negative, viral RNA may be detected in LRT secretions, such as sputum or bronchoalveolar lavage [70, 71, 75, 76].  Faeces or rectal swabs have been shown to be positive for SARS-CoV-2 RNA in a subset of patients, with some studies suggesting that this positivity is prolonged compared to that of respiratory tract specimens [46, 56, 59, 75, 77]. In some patients, SARS-CoV-2 RNA detection in blood samples has been reported and some studies suggest that detection in the blood is associated with disease severity, however, more studies on this potential association are required [75, 78-81]. In oral fluid specimens (e.g. induced saliva) [28, 49, 82-88], reported detection rates compared with URT specimens from the same patient vary widely, and limited data are available on adequacy of SARS-CoV-2 detection in gargling/mouth washes [85]. The striking differences in sensitivity of oral fluids evaluations are potentially due to large differences in collection, transport and storage techniques, as well as the evaluation of different testing populations. Occasionally, SARS-CoV-2 can be detected in ocular fluids in patients with and without signs of conjunctivitis [89-93]. Some studies have not detected SARS-CoV-2 in urine [58, 75, 94], while others were able to detect viral RNA in urine in a limited number of patients [57, 95].  One study reported several patients with positive semen samples [96]. In addition, positive RNA detection for brain tissue [97] and cerebrospinal fluid [98] have been described in case reports. Thus, SARS-CoV-2 can be detected in a wide range of other body fluids and compartments, but it is most frequently detected in respiratory material and, therefore, respiratory samples remain the sample type of choice for diagnostics.

## Laboratory testing guiding principles

The decision to test should be based on both clinical and epidemiological factors. See the interim guidance clinical management of COVID-19 [99], investigations of clusters  [6] and public health surveillance [7].

Rapid collection of appropriate specimens from and accurate laboratory diagnosis of patients in whom SARS-CoV-2 infection is strongly suspected are the two priorities to support clinical management of patients and infection control measures. Given the complexity of adequate sampling, laboratory analysis, and interpretation of results, collection and laboratory diagnosis should be performed by trained and competent operators.

Individuals infected with SARS-CoV-2 may never develop symptoms (asymptomatic cases), they may have very mild disease (pauci-symptomatic), or they may develop moderate to severe COVID-19 disease [18-26]. The most robust evidence for viral infection comes from the detection of fragments of the virus, such as proteins or nucleic acids, through virological testing. Infected individuals may test positive for viral nucleic acids or viral proteins without symptoms (asymptomatic), or before symptom onset (pre-symptomatic), and throughout a disease episode (symptomatic). For those who develop COVID-19 illness, symptoms can be wide-ranging at initial presentation of disease. Individuals may present with very mild symptoms, with apparent pneumonia, febrile illnesses/sepsis, and less commonly with gastro-enteritis or neurological symptoms [99]. If required for case management, patients should also be tested for other pathogens, as recommended in local clinical management guidelines, but this should never delay testing for SARS-CoV-2 [99, 100]. Co-infections of SARS-CoV-2 with other pathogens have been reported, thus a positive test for another pathogen does not rule out COVID-19 and vice versa [27, 101-109]. Cases of false positive dengue antibody test results using a dengue rapid diagnostic test (RDT) in COVID-19 patients have been reported [110, 111]. There is also a risk of false positive or false negative SARS-CoV-2 results, if testing is not performed with adequate assays or not done under adequate conditions.

# Specimen collection, shipment and storage

**Safety procedures during specimen collection**

Ensure that health workers collecting clinical specimens from suspect cases adhere rigorously to infection prevention and control guidelines (IPC) and wear appropriate personal protective equipment (PPE), see also COVID-19 WHO interim guidance on infection prevention and control during health care [7].

Ensure that adequate standard operating procedures (SOP) are in place and that staff are appropriately trained in specimen collection, packaging, shipment and storage. It should be assumed that all specimens collected for investigations may be infected with SARS-CoV-2 and other pathogens. See also WHO interim guidance on laboratory biosafety for SARS-CoV-2 [3]. Local guidelines, including on informed consent, should be followed for specimen collection, testing, storage and research.

**Specimens to be collected**

The optimal specimen depends on clinical presentation and time since symptom onset. At minimum, respiratory specimens should be collected.

Respiratory specimens

- **Upper respiratory specimens** are adequate for testing early-stage infections, especially in asymptomatic or mild cases. Testing combined nasopharyngeal and oropharyngeal swabs from one individual has been shown to increase sensitivity for detection of respiratory viruses and improve the reliability of the result [60, 86, 112-114]. Two individual swabs can be combined in one collection tube or a combined nasopharyngeal and oropharyngeal swab can be taken [115]. A few studies have found that individual nasopharyngeal swabs yield a more reliable result than oropharyngeal swabs [40, 75, 76, 114].

- **Lower respiratory specimens** are advised if collected later in the course of the COVID-19 disease or in patients with a negative URT sampling and there is a strong clinical suspicion of COVID-19 [70, 71, 75, 76, 86]. LRT specimens can consist of sputum, if spontaneously produced (induced sputum is not recommended as this poses an increased risk of aerosol transmission [99]) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. Caution should be exercised due to the high risk of aerosolization; therefore strict adherence to IPC procedures during sample collection is required. The indication for an invasive procedure should be evaluated by a physician.

Before implementing other respiratory or oral fluid sampling methods, the sampling method should first pass validation in the laboratory for the intended patient groups.

Simplified and optimized specimen collection

There is a high demand for simplified and optimized specimen collection for SARS-CoV-2 detection. Studies on combined oropharyngeal and nares/nasal swab [116, 117], others on midturbinate [118-120] or lower nasal or nares swabs [120, 121] or tongue swab [120] either by a trained sampler or by self-sampling have been conducted. While some of these studies show that these approaches perform reasonably well, these studies focus mostly on specific patient groups and their sample sizes are limited. Before broad implementation of these alternatives can be recommended, further assessment and validation is needed to determine the indications for which these collection methods serve as appropriate alternatives.

There are specific cases where collecting nasopharyngeal and oropharyngeal swabs can be problematic, such as mass screening in schools or nursing homes, especially when elderly people with dementia or young children are involved. In these scenarios, oral fluids could potentially be a suitable specimen, as the collection methods are less invasive and there is a lower risk of exposure to others upon collection, as compared with the collection of URT specimens.

Oral fluid collection methods vary widely: from posterior oropharyngeal fluids/saliva collected by spitting or drooling, or collection of oral fluid with pipet or special sponges. Gargling with saline solutions is another alternative that has been studied. Sensitivity of these specimens has a wide performance range compared with naso- and/or oropharyngeal sampling [28, 49, 82, 83, 85-88, 122-125]. Due to the large variety of collection methods and processing steps, laboratories must collect their own performance data linked to the local method of collection and in the relevant population for testing. At this time, WHO does not recommend the use of saliva as the sole specimen type for routine clinical diagnostics. If nonstandard collection methods are intended to be used to diagnose other respiratory pathogens, the detection of these pathogens needs to be part of the validation procedure.

Faecal specimens

From the second week after symptom onset and onwards, NAAT can be considered for faecal specimens in cases where URT and LRT are negative and the clinical suspicion of a COVID-19 infection remains [126]. When testing faeces, ensure that the intended extraction method and NAAT has been validated for this type of sample.

Post-mortem specimens

If the person is deceased, consider taking a post-mortem swab, needle biopsy or tissue specimens from the autopsy, including lung tissue for further pathological and microbiological testing [127-133].

<u>Serum specimens</u>

If negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2-4 weeks later can be used to look for seroconversion or a rise in antibody titres. These two samples can be used retrospectively to determine whether the individual has had COVID-19, especially when the infection could not be detected using NAAT.

See Figure 1 for the diagnostic algorithm for cases requiring clinical care and are suspected to have COVID-19.

*Figure 1: Diagnostic flow diagram for the detection of acute SARS-CoV-2 infection in individuals with clinical suspicion for COVID-19*
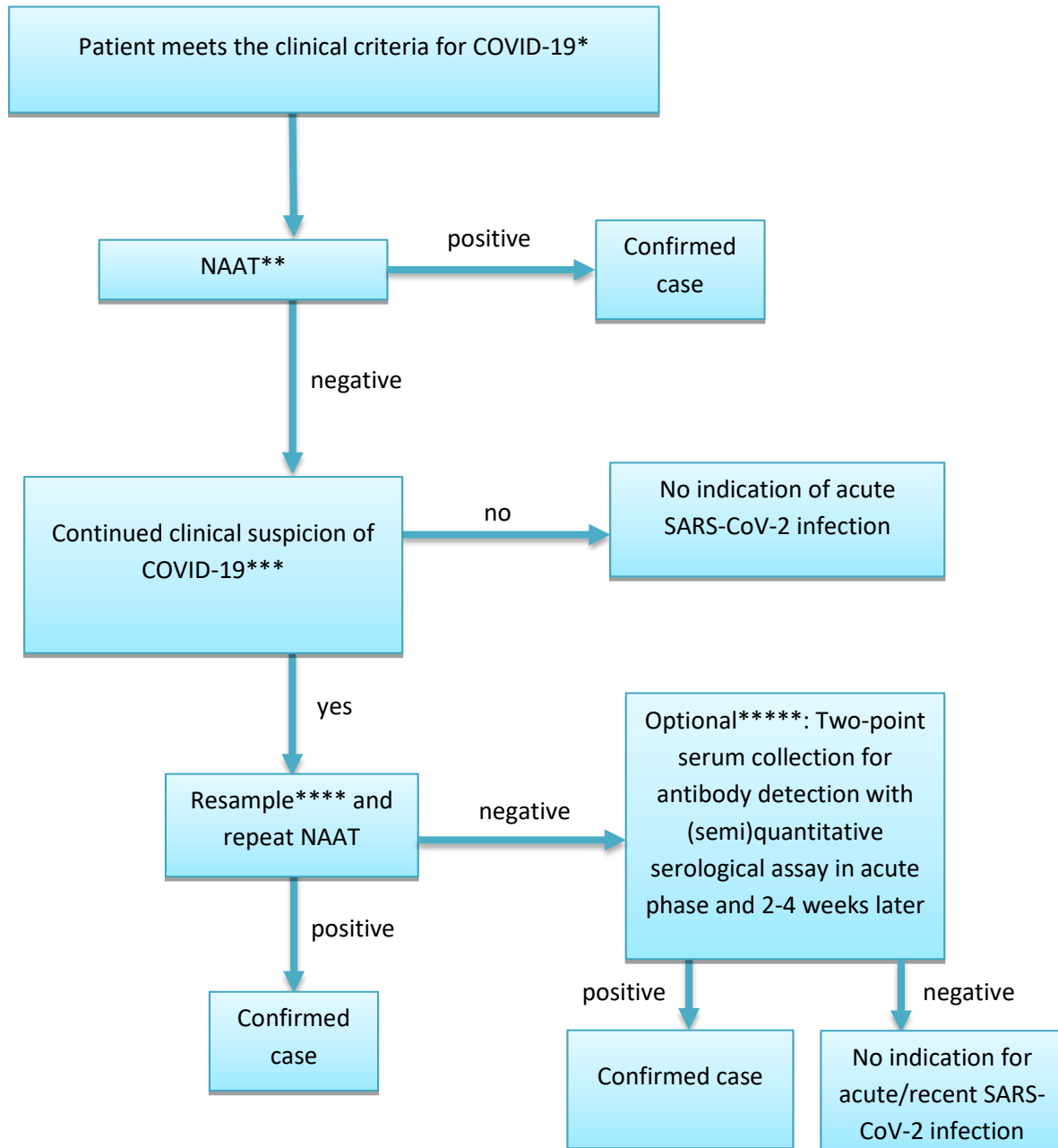


* Clinical management of COVID-19 (Interim Guidance), World Health Organization [99].
** If antigen detection would be incorporated into the testing algorithm, how this needs to be done depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS-CoV-2 infection in the intended testing population. For more information see section below on "Rapid diagnostic tests based on antigen detection" and the specific guidance Interim guidance on antigen-detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5].
*** Continued clinical suspicion can, for example, be the absence of another obvious etiology, the presence of an epidemiological link, or suggestive clinical finding (e.g. typical radiological signs).
**** The selection of specimen type will depend on the clinical presentation, see section "Specimens to be collected". Increasing the number of samples tested will also increase the sensitivity of testing for COVID-19. More than two samples might be needed on some occasions to detect SARS-CoV-2 [73].
***** For interpretation of serology, see section "Implementation and interpretation of antibody testing in the clinical laboratory". Serology cannot be used as a standalone diagnostic for acute SARS-CoV-2 infections and clinical management.

**Packaging and shipment of clinical specimens**

Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation and in the laboratory is essential. For guidance on this see Annex 1.

Transportation of specimens within national borders should comply with the applicable national regulations. International transport of specimens that may contain SARS-CoV-2 should follow the United Nations Model Regulations, Biological Substance, Category B (UN 3373), and any other applicable regulations depending on the mode of transport.

More information may be found in the WHO Guidance on regulations for the transport of infectious substances 2019-2020 [134] and specific SARS-CoV-2  laboratory biosafety guidance [3] and shipment instructions [135].

Maintain open and efficient lines of communication with the laboratory and provide all requested information. Specimens should be correctly labelled and accompanied by a diagnostic request form (see Annex 2 for a request form template, including minimal required clinical information). Alerting the laboratory before sending specimens and providing the essential background information with the diagnostic request allows for the proper and timely processing of specimens and reporting of results.

## Biosafety practices in the laboratory

Laboratories undertaking testing for SARS-CoV-2 should adhere strictly to the appropriate biosafety practices. Testing clinical specimens that may contain SARS-CoV-2 should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances. Specimen handling for molecular testing using standard rRT-PCR requires biosafety level (BSL) 2 or equivalent facilities with the use of a biosafety cabinet (BSC) or a primary containment device that is recommended for sample manipulation before inactivation.

Attempts to isolate the virus in cell culture require BSL-3 facilities at a minimum. When performing viral culture on potentially SARS-CoV-2 positive clinical specimens for other purposes, a risk assessment needs to be conducted followed by required safety measures and procedures [136].

Specific considerations of biosafety requirements may allow certain point-of-care (POC) or near-patient assays to be performed outside a biosafety cabinet, once local regulations have been reviewed, after performing risk assessment and having put in place adequate risk-mitigation measures. For further details on laboratory biosafety, see specific laboratory biosafety interim guidance [3]. For general laboratory biosafety guidelines, see the WHO Laboratory biosafety manual, 3rd edition [136].

## Testing for SARS-CoV-2

**Nucleic acid amplification test (NAAT)**

Wherever possible, suspected active SARS-CoV-2 infections should be tested with NAAT, such as rRT-PCR. NAAT assays should target the SARS-CoV-2 genome. Since there is currently no known circulation of SARS-CoV-1 globally, a sarbecovirus-specific sequence is also a reasonable target. For commercial assays, interpretation of results should be done according to the instructions for use. Optimal diagnostics consist of a NAAT assay with at least two independent targets on the SARS-CoV-2 genome, however, in areas with widespread transmission of SARS-CoV-2, a simple algorithm might be adopted with one single discriminatory target. When using a one-target assay, it is recommended to have a strategy in place to monitor for mutations that might affect performance. For more details, see section below on "Background information on monitoring for mutations in primer and probe regions".

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**Background information on monitoring for mutations in primer and probe regions**

As SARS-CoV-2 continues to acquire genetic changes over time, mismatches between primers and/or probes, and corresponding binding sites within SARS-CoV-2 genomes may reduce NAAT sensitivity. Where feasible, monitor for primer and probe mismatches due to SARS-CoV-2 mutations, and assess their impact. By routinely testing all specimens with two different primer/probe sets that target different genomic regions it is possible to reduce the risk of false-negative results. Several tools monitoring for relevant mutations are available, including searches done by GISAID (the Global Initiative on Sharing All Influenza Data) and other tools including PrimerCheck (Erasmus Medical Centre), PrimerScan (European Centre for Disease prevention and Control) and CoV-GLUE (COVID-19 UK Genomics Consortium and MRC-University of Glasgow Centre for Virus Research). Primercheck and COV-GLUE allows researchers to use their own sequence data confidentially as input. Not all mutations in primer/probe regions lead to significant changes in performance. *In silico* predictions of binding efficiency are insufficient to quantify the effect of a mismatch on the sensitivity of a NAAT, so it is essential to do an experimental comparison of the assay's sensitivity for both variant and reference virus isolates. For commercial assays, it is vital to keep track of possible incidents of suboptimal performance. Inform the manufacturer of the assay and WHO of any concerns you may experience with a specific assay.

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Many in-house and commercial rRT-PCR assays have become available and several have been independently validated [137-143]. Some considerations for selecting the right NAAT for the laboratory are listed in Annex 3. A few of the NAAT systems have the capacity for fully automated testing that integrates sample processing as well as the capacity for RNA extraction, amplification and

reporting. Such systems provide access to testing in locations with limited laboratory capacity and rapid turnaround time when used for near-patient testing. Validation data of some of these assays are now available [144]. When implementing these assays in specific settings, staff performing the test should be appropriately trained, performance should be assessed in those specific settings and a system to monitor quality should be put in place. Additional potentially valuable amplification/detection methods, such as CRISPR (targeting clustered regularly interspaced short palindromic repeats), isothermal nucleic acid amplification technologies (e.g. reverse transcription loop-mediated isothermal amplification (RT-LAMP), and molecular microarray assays are under development  or in the process of being commercialized [145-147]. Validation of the analytic and clinical performance of these assays, demonstration of their potential operational utility, rapid sharing of data, as well as emergency regulatory review of manufacturable, well-performing tests are encouraged to increase access to SARS-CoV-2 testing.

Careful interpretation of weak positive NAAT results is needed, as some of the assays have shown to produce false signals at high Ct values. When test results turn out to be invalid or questionable, the patient should be resampled and retested.  If additional samples from the patient are not available, RNA should be re-extracted from the original samples and retested by highly experienced staff. Results can be confirmed by an alternative NAAT test or via virus sequencing if the viral load is sufficiently high. Laboratories are urged to seek reference laboratory confirmation of any unexpected results.

One or more negative results do not necessarily rule out the SARS-CoV-2 infection [40, 42, 58, 66-74]. A number of factors could lead to a negative result in an infected individual, including:

- poor quality of the specimen, because it contains too little patient material;

- the specimen was collected late in the course of the disease, or the specimen was taken from a body compartment that did not contain the virus at that given time;

- the specimen was not handled and/or shipped appropriately;

- technical reasons inherent in the test, e.g. PCR inhibition or virus mutation.

For clinical case management a proposed testing algorithm is depicted in Figure 1.

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**Alternatives to RNA extraction**

Most conventional molecular diagnostic workflows require RNA extraction before an rRT-PCR test is conducted. However, there are global shortages of commercial extraction kits due to the COVID-19 pandemic. Direct rRT-PCR from nasopharyngeal swabs may provide an emergency or temporary alternative to RNA extraction, but limitations to the input volume, as well as an increased risk of RNA degradation and PCR inhibition can lead to a loss of sensitivity of the assay [148, 149]. Heat treatment prior to sample processing can affect the RNA quality [149, 150]. Other factors that can affect RNA quality and which should be evaluated before implementation are the addition of detergents, transport media, the volume of the specimen used, and the polymerase enzyme used [148, 151-154]. The biosafety implications of alternative extraction workflows should also be considered. Laboratories considering alternative methods that bypass the need for RNA extraction should validate their protocols thoroughly and conduct a risk assessment that weighs the benefits and risks, before integrating such protocols into a diagnostic workflow.

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**Pooling of specimens for NAAT**

Pooling of samples from multiple individuals can increase the diagnostic capacity for detecting SARS-CoV-2 when the rate of testing does not meet the demand in some settings [155-159]. There are several strategies for pooling specimens. If the pooled result is negative, all individual specimens in the pool are regarded as negative. If the pool tests positive the follow-up steps depend on the strategy, but in general each specimen needs to undergo individual testing (pool deconvolution) to identify the positive specimen(s). Another approach is matrix pooling. This means that pools are made per row and per column, and tested by PCR, the position in the matrix identifies the positive specimen without additional testing if prevalence is sufficiently low. Depending how robust the matrix testing method is in the specific context, it might still be advisable to retest the identified positive samples for confirmation. Pooling of specimens could be considered in population groups with a low/very low expected prevalence of SARS-CoV-2 infection, but not for cases or cohorts that more likely to be infected with SARS-CoV-2. Routine use of the pooling of specimens from multiple individuals in clinical care and for contact tracing purposes is not recommended. Studies have been conducted to determine the optimal sample pooling number and design pooling strategies in different outbreak settings [156, 160-162].

Before any sample pooling protocols can be implemented, they must be validated in the appropriate populations and settings. An inappropriate testing strategy may lead to missed cases or other laboratory errors that may, in turn, negatively affect patient management and public health control measures. In addition, the risk of cross-contamination and the potential increase in workload complexity and volume must be considered. To perform reliable pooling, adequate automation is key (e.g. robotic systems, software supporting the algorithms to identify positive samples, laboratory information systems and middle-ware that can work with sample pooling).

Based on currently available data, intra-individual pooling (multiple specimens from one individual that are pooled and tested as a single sample) from upper respiratory tract samples can be used. Intra-individual pooling of sputum and faeces with URT samples is not recommended because the former may contain compounds that inhibit rRT-PCR.

**Rapid diagnostic tests based on antigen detection**

Rapid diagnostic tests that detect the presence of SARS-CoV-2 viral proteins (antigens) in respiratory tract specimens are being developed and commercialized. Most of these are lateral flow immunoassays (LFI), which are typically completed within 30 minutes. In contrast to NAATs, there is no amplification of the target that is detected, making antigen tests less sensitive. Additionally, false-positive (indicating that a person is infected when they are not) results may occur if the antibodies on the test strip also recognize antigens of viruses other than SARS-CoV-2, such other human coronaviruses.

The sensitivity of different RDTs compared to rRT-PCR in specimens from URT (nasopharyngeal swabs) appears to be highly variable [144, 163-165], but specificity is consistently reported to be high. Currently, data on antigen performance in the clinical setting is still limited: paired NAAT and antigen validations in clinical studies are encouraged to identify which of the antigen detection tests that are either under development or have already been commercialized demonstrate acceptable performance in representative field studies. When performance is acceptable, antigen RDTs could be implemented in a diagnostic algorithm to reduce the number of molecular tests that need to be performed and to support rapid identification and management of COVID-19 cases. How antigen detection would be incorporated into the testing algorithm depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS-CoV-2 infection in the intended testing population. Higher viral loads are associated with improved antigen test performance; therefore test performance is expected to be best around symptom onset and in the initial phase of a SARS-CoV-2 infection. For specific guidance on antigen detection tests see the WHO Interim guidance on antigen-detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5].

**Antibody testing**

Serological assays that detect antibodies produced by the human body in response to infection with the SARS-CoV-2 can be useful in various settings.

For example, serosurveillance studies can be used to support the investigation of an ongoing outbreak and to support the retrospective assessment of the attack rate or the size of an outbreak [9]. As SARS-CoV-2 is a novel pathogen, our understanding of the antibody responses it engenders is still emerging and therefore antibody detection tests should be used with caution, and not used to determine acute infections.

Non-quantitative assays (e.g. lateral flow assays) cannot detect an increase in antibody titres, in contrast to (semi)quantitative or quantitative assays. Lateral flow antibody detection assays (or other non-quantitative assays) are currently not recommended for acute diagnosis and clinical management and their role in epidemiologic surveys is being studied. For more information on the utility of rapid immunodiagnostic tests, we refer to the WHO scientific brief with advice on the specific SARS-CoV-2 point-of-care immunodiagnostic tests [4].

Serology should not be used as a standalone diagnostic to identify acute cases in clinical care or for contact tracing purposes. Interpretations should be made by an expert and are dependent on several factors including the timing of the disease, clinical morbidity, the epidemiology and prevalence within the setting, the type of test used, the validation method, and the reliability of the results.

Seroconversion (development of measurable antibody response after infection) has been observed to be more robust and faster in patients with severe disease compared to those with milder disease or asymptomatic infections. Antibodies have been detected as early as in the end of the first week of illness in a fraction of patients, but can also take weeks to develop in patients with subclinical/mild infection [37, 166-173]. A reliable diagnosis of COVID-19 infection based on patients' antibody response will often only be possible in the recovery phase, when opportunities for clinical intervention or interruption of disease transmission have passed. Therefore, serology is not a suitable replacement for virological assays to inform contact tracing or clinical management. The duration of the persistence of antibodies generated in response to SARS-CoV-2 is still under study [49, 174]. Furthermore, the presence of antibodies that bind to SARS-CoV-2 does not guarantee that they are neutralizing antibodies, or that they offer protective immunity.

**Available serological tests for detecting antibodies**

Commercial and non-commercial tests measuring binding antibodies (Total immunoglobulins (Ig), IgG, IgM, and/or IgA in different combinations) utilizing various techniques including LFI, enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA) have become available. A number of validations and systematic reviews on these assays have been published [170, 171, 173, 175-177]. The performance of serologic assays varies widely in different testing groups (such as in patients with mild versus moderate-to-severe disease as well as in young versus old), timing of testing and the target viral protein. Understanding these performance variations will require further study. Antibody detection tests for coronavirus may also cross-react with other pathogens, including other human coronaviruses, [167, 178-180] or with pre-existing conditions (e.g. pregnancy, autoimmune diseases) and thus yield false-positive results.

Virus neutralization assays are considered to be the gold standard test for detecting the presence of functional antibodies. These tests require highly skilled staff and BSL-3 culture facilities and, therefore, are unsuitable for use in routine diagnostic testing.

**Implementation and interpretation of antibody testing in the clinical laboratory**

When implementing serological assays in the clinical laboratory, an in-house validation or verification of the specific assays is advisable. Even if commercial tests have been authorized for use in emergencies, an in-house verification (or if required by local authorities a validation) is still required. Protocols and examples with suggestions as to how to do this are now available [170, 171, 181].

Each serological test is different. With regard to commercial tests, follow the manufacturer's instructions for use. Studies show that several commercial assays measuring total Ig or IgG have performed well. Most of these studies show no advantage of IgM over IgG, as IgM does not appear much earlier than IgG [173]. The additional role of IgA testing in routine diagnostics has not been established. For confirmation of a recent infection, acute and convalescent sera must be tested using a validated (semi)quantitative or quantitative assay. The first sample should be collected during the acute phase of illness, and the second sample at least 14 days after the initial sera was collected. Maximum antibody levels are expected to occur in the third/fourth week after symptom onset. Seroconversion or a rise in antibody titres in paired sera will help to confirm whether the infection is recent and/or acute. If the initial sample tests positive, this result could be due to a past infection that is not related to the current illness.

The first known case of reinfection with SARS-CoV-2 has been documented [182]. Only limited information is available on the interpretation of SARS-CoV-2 antibody tests after a previous infection with SARS-CoV-2 and on the dynamics of SARS-CoV-2 serology if a subsequent infection with another coronavirus occurs. In these two sets of circumstances interpretation of serology may be extremely challenging.

**Viral isolation**

Virus isolation is not recommended as a routine diagnostic procedure. All procedures involving viral isolation in cell culture require trained staff and BSL-3 facilities. A thorough risk assessment should be carried out when culturing specimens from potential SARS-CoV-2 patients for other respiratory viruses because SARS-CoV-2 has been shown to grow on a variety of cell lines [183].

**Genomic sequencing for SARS-CoV-2**

Genomic sequencing for SARS-CoV-2 can be used to investigate the dynamics of the outbreak, including changes in the size of an epidemic over time, its spatiotemporal spread, and testing hypotheses about transmission routes. In addition, genomic sequences can be used to decide which diagnostic assays, drugs and vaccines may be suitable candidates for further exploration. Analysis of SARS-CoV-2 virus genomes can, therefore, complement, augment and support strategies to reduce the disease burden of COVID-19. However, the potentially high cost and volume of the work required for genomic sequencing means that laboratories should have clarity about the expected returns from such investment and what is required to maximize the utility of such genomic sequence data. WHO guidance on SARS-CoV-2 genomic sequencing is currently being developed.

**Quality assurance**

Before introducing a new testing method, a new assay, new batches of materials, or a new PCR technician into the laboratory, a validation or verification should be carried out, to ensure that the laboratory testing system is performing adequately.

For manual PCR systems, each NAAT sample should include internal controls and ideally a specimen collection control (human gene target). Additionally, external controls are recommended for each test run. Laboratories that order their own primers and probes should carry out entry testing or validation looking at functionality and potential contaminants [184].

Laboratories are encouraged to define their assays' detection limits, and senior staff should recognize how disease prevalence alters the predictive value of their test results. Once the number of cases goes down, the positive predictive value will decrease, therefore