

Diagnostic testing for SARS-CoV-2

Interim guidance

11 September 2020



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.

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

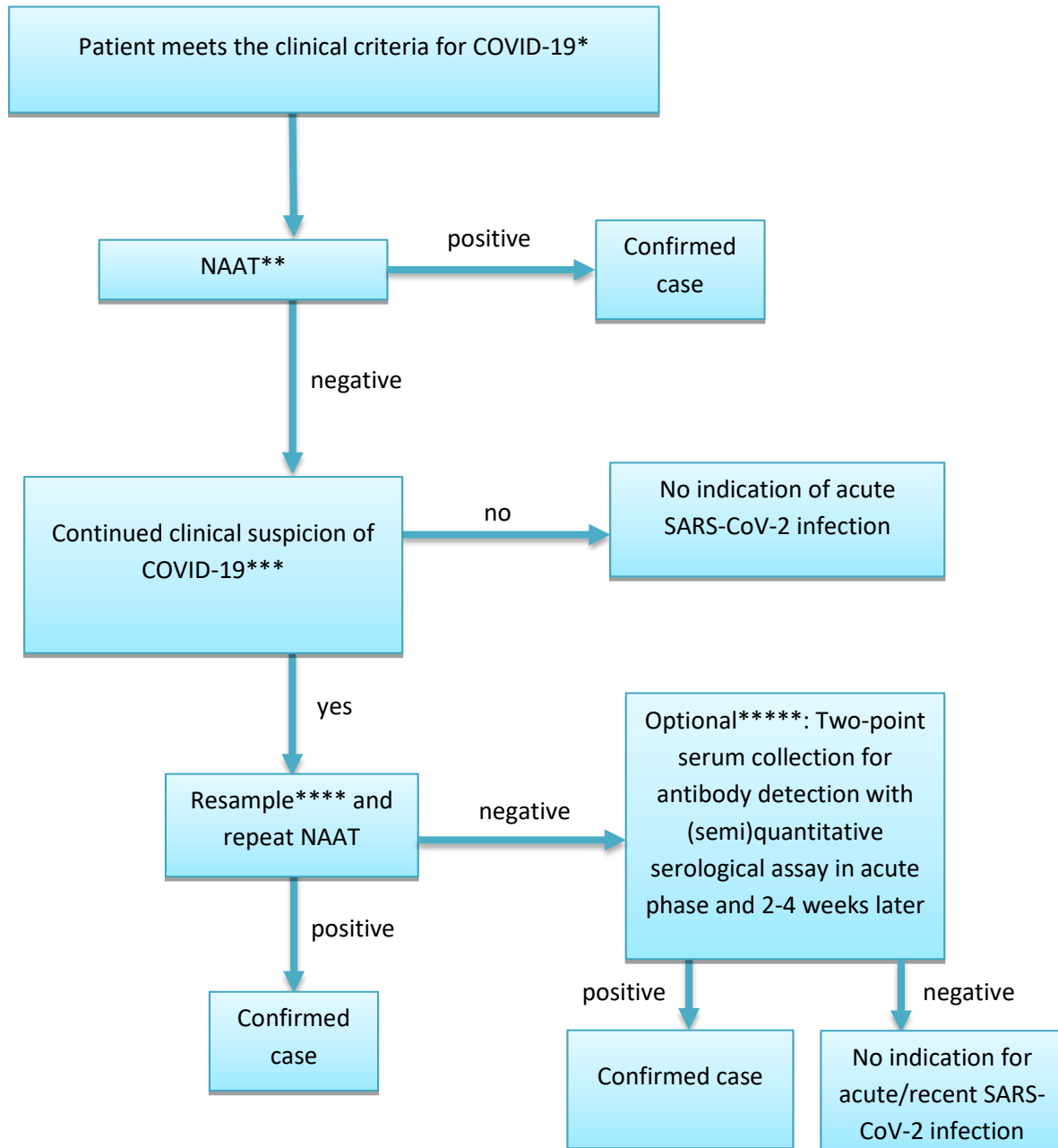
If the person is deceased, consider taking a post-mortem swab, needle biopsy or tissue specimens from the autopsy, including lung tissue for further pathological and microbiological testing [127-133].

Serum specimens

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”.

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.

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.

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.

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 as 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 the interpretation of tests should continue to be part of a stringent quality assurance scheme, with interpretation based on: timing of sampling, sample type, test specifics, clinical data and epidemiological data.

Laboratories should put measures in place to reduce the potential for false positive rRT-PCR results and have a strategy for the management of equivocal results. See Annex 4 for a checklist.

In general, laboratories should have a quality assurance system in place and are encouraged to participate in external quality assessment (EQA) schemes or perform result comparison between laboratories of a subset of samples.

WHO has previously advised national laboratories to ensure quality performance by confirmation of testing results for the first 5 positive specimens and the first 10 negative specimens (collected from patients that fit the case definition) by referring them to one of the WHO reference laboratories that provide confirmatory testing for SARS-CoV-2. WHO provided support to national laboratories to facilitate specimen shipment to one of the dedicated reference laboratories. For more information, consult the WHO website for [the list of reference laboratories](#) [185] and [shipment instructions](#) [135]. Strengthened national reference laboratories and growing access to EQA schemes for SARS-CoV-2 reduce the need to use this mechanism. If testing for SARS-CoV-2 is not yet available in a country, efforts should be made to establish national capacity.

Reporting of cases and test results

Rapid communication of test results is important for planning and design of public health and outbreak control interventions. Laboratories should follow national reporting requirements. In general, all test results, positive or negative, should be immediately reported to the national authorities. States Parties to the International Health Regulations (IHR) are reminded of their obligation to share with WHO relevant public health information for events for which they must notify WHO, using the decision instrument in Annex 2 of the IHR (2005) [186].

Regular interaction between public health experts, clinicians and local laboratory experts to discuss strategies, potential problems and solutions, should be considered to be an essential part of an adequate COVID-19 response. This response includes the development of guidance and (clinical-, epidemiological-, and trial) study protocols.

A rapid turnaround time of test results can, in turn, have a positive impact on the outbreak [187, 188]. More studies are needed to fine tune the maximum acceptable time from symptom onset to sample result to have impact on clinical management and outbreak control; currently a maximum of 24 hours is considered reasonable in most settings. As laboratories often have control only over the time between sample arrival and the test result, it is critical to ensure that samples arrive in the laboratory without delay.

Methods

This document was developed in consultation with experts from the SARS-CoV-2 laboratory expert network. Experts in the network completed a confidentiality agreement and declaration of interest. The declaration of interest forms were reviewed, and no conflicts regarding the support of this guidance document were identified. Relevant WHO guidance has been used in this document [136, 185, 189-194]. This is the sixth edition (version 2020.6) and was originally adapted from *Laboratory testing for Middle East Respiratory Syndrome Coronavirus* [189].

A broad spectrum of clinical laboratory experts from different regions were engaged in the development of this document. The internal experts involved in the development include WHO regional laboratory focal points, epidemiologists and clinical experts. This version of the guidance incorporates the novel understanding and characteristics of the virus and addresses questions and issues received from WHO's country and regional offices and other channels.

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References

1. *Laboratory testing strategy recommendations for COVID-19*. World Health Organization 2020; Available from: <https://apps.who.int/iris/handle/10665/331509>.
2. *Laboratory assessment tool for laboratories implementing COVID-19 virus testing*. World Health Organization 2020 [8 April 2020 7 July 2020]; Available from: <https://apps.who.int/iris/handle/10665/331715>.
3. *Laboratory biosafety guidance related to coronavirus disease (COVID-19)*. World Health Organization 2020; Available from: <https://apps.who.int/iris/handle/10665/332076>
4. *Advice on the use of point-of-care immunodiagnostic tests for COVID-19*. World Health Organization 8 April 2020; Available from: <https://apps.who.int/iris/handle/10665/331713>.
5. *Antigen detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays, interim guidance*. World Health Organization 2020. Available from: <https://apps.who.int/iris/handle/10665/334253>

6. *Considerations in the investigation of cases and clusters of COVID-19*. World Health Organization 2020; Available from: <https://apps.who.int/iris/handle/10665/331668>.
7. *Public health surveillance for COVID-19: interim guidance*,. 7 August 2020; Available from: <https://apps.who.int/iris/handle/10665/333752>.
8. *Operational considerations for COVID-19 surveillance using GISRS, interim guidance*. World Health Organization 2020; Available from: <https://apps.who.int/iris/handle/10665/331589>.
9. *The Unity Studies: Early Investigations Protocols*. 2020 [27 July 2020]; Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/early-investigations>.
10. Gorbalenya A, B.S., Baric R, de Groot R, Drosten C, Gulyaeva A, Haagmans B, Lauber C, Leontovich A, Neuman B, Penzar D, Perlman S, Poon L, Samborskiy D, Sidorov I, Sola I, Ziebuhr J, *The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2*. Nat Microbiol, 2020. **5**(4): p. 536-544.
11. *International Committee on Taxonomy of Viruses (ICTV)*. 2020 [27 July 2020]; Available from: <https://talk.ictvonline.org/>.
12. Naqvi, A.A.T., et al., *Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach*. Biochim Biophys Acta Mol Basis Dis, 2020. **1866**(10): p. 165878.
13. Yoshimoto, F.K., *The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COV19), the Cause of COVID-19*. Protein J, 2020. **39**(3): p. 198-216.
14. Kim, D., et al., *The Architecture of SARS-CoV-2 Transcriptome*. Cell, 2020. **181**(4): p. 914-921 e10.
15. Lu, R., et al., *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding*. Lancet, 2020. **395**(10224): p. 565-574.
16. Yan, R., et al., *Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2*. Science, 2020. **367**(6485): p. 1444-1448.
17. Ni, W., et al., *Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19*. Crit Care, 2020. **24**(1): p. 422.
18. Wu, Z. and J.M. McGoogan, *Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention*. JAMA, 2020.
19. Mizumoto, K., et al., *Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020*. Euro Surveill, 2020. **25**(10).
20. He, J., et al., *Proportion of asymptomatic coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis*. J Med Virol, 2020.
21. Kronbichler, A., et al., *Asymptomatic patients as a source of COVID-19 infections: A systematic review and meta-analysis*. Int J Infect Dis, 2020.
22. Al-Sadeq, D.W. and G.K. Nasrallah, *The incidence of the novel coronavirus SARS-CoV-2 among asymptomatic patients: a systematic review*. Int J Infect Dis, 2020.
23. Kluytmans-van den Bergh, M.F.Q., et al., *Prevalence and Clinical Presentation of Health Care Workers With Symptoms of Coronavirus Disease 2019 in 2 Dutch Hospitals During an Early Phase of the Pandemic*. JAMA Netw Open, 2020. **3**(5): p. e209673.
24. Gudbjartsson, D.F., et al., *Spread of SARS-CoV-2 in the Icelandic Population*. N Engl J Med, 2020. **382**(24): p. 2302-2315.
25. Arons, M.M., et al., *Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility*. N Engl J Med, 2020.
26. Bitnun, A., et al., *Severe acute respiratory syndrome-associated coronavirus infection in Toronto children: a second look*. Pediatrics, 2009. **123**(1): p. 97-101.
27. Richardson, S., et al., *Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area*. JAMA, 2020.
28. Wyllie, A.L., et al., *Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2*. N Engl J Med, 2020.
29. WHO. *R&D blueprint and COVID-19*. 2020 [cited 2020 16 July 2020]; Available from: <https://www.who.int/teams/blueprint/covid-19>.
30. CLOPID-R. *Global research collaboration for infectious disease preparedness, preparedness, data sharing*. 2020 [16 July 2020]; Available from: <https://www.glopid-r.org/our-work/data-sharing/>.
31. Li, Q., et al., *Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia*. N Engl J Med, 2020. **382**(13): p. 1199-1207.
32. Guan, W.J., et al., *Clinical Characteristics of Coronavirus Disease 2019 in China*. N Engl J Med, 2020. **382**(18): p. 1708-1720.
33. Linton, N.M., et al., *Incubation Period and Other Epidemiological Characteristics of 2019 Novel Coronavirus Infections with Right Truncation: A Statistical Analysis of Publicly Available Case Data*. J Clin Med, 2020. **9**(2).

34. Lauer, S.A., et al., *The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application*. Ann Intern Med, 2020. **172**(9): p. 577-582.
35. Backer, J.A., D. Klinkenberg, and J. Wallinga, *Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020*. Euro Surveill, 2020. **25**(5).
36. He, X., et al., *Temporal dynamics in viral shedding and transmissibility of COVID-19*. Nat Med, 2020. **26**(5): p. 672-675.
37. Wolfel, R., et al., *Virological assessment of hospitalized patients with COVID-2019*. Nature, 2020.
38. Weiss, A., M. Jellingso, and M.O.A. Sommer, *Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: A systematic review and meta-analysis*. EBioMedicine, 2020. **58**: p. 102916.
39. Sethuraman, N., S.S. Jeremiah, and A. Ryo, *Interpreting Diagnostic Tests for SARS-CoV-2*. JAMA, 2020.
40. Zou, L., et al., *SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients*. N Engl J Med, 2020. **382**(12): p. 1177-1179.
41. Wang, Y., et al., *Kinetics of viral load and antibody response in relation to COVID-19 severity*. J Clin Invest, 2020.
42. Young, B.E., et al., *Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore*. JAMA, 2020.
43. Kam, K.Q., et al., *A Well Infant with Coronavirus Disease 2019 (COVID-19) with High Viral Load*. Clin Infect Dis, 2020.
44. Hu, Z., et al., *Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China*. Sci China Life Sci, 2020. **63**(5): p. 706-711.
45. Liu, Y., et al., *Viral dynamics in mild and severe cases of COVID-19*. Lancet Infect Dis, 2020.
46. Zheng, S., et al., *Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study*. BMJ, 2020. **369**: p. m1443.
47. Lavezzo, E., et al., *Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'*. Nature, 2020. **Nature**.
48. Agnihothram, S., et al., *Evaluation of serologic and antigenic relationships between middle eastern respiratory syndrome coronavirus and other coronaviruses to develop vaccine platforms for the rapid response to emerging coronaviruses*. J Infect Dis, 2014. **209**(7): p. 995-1006.
49. To, K.K., et al., *Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study*. Lancet Infect Dis, 2020. **20**(5): p. 565-574.
50. Li, N., X. Wang, and T. Lv, *Prolonged SARS-CoV-2 RNA shedding: Not a rare phenomenon*. J Med Virol, 2020.
51. Zhou, B., et al., *The duration of viral shedding of discharged patients with severe COVID-19*. Clin Infect Dis, 2020.
52. Chen, Y., et al., *The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients*. J Med Virol, 2020.
53. Gupta, S., et al., *Persistent viral shedding of SARS-CoV-2 in faeces - a rapid review*. Colorectal Dis, 2020.
54. Xu, K., et al., *Factors associated with prolonged viral RNA shedding in patients with COVID-19*. Clin Infect Dis, 2020.
55. Qi, L., et al., *Factors associated with duration of viral shedding in adults with COVID-19 outside of Wuhan, China: A retrospective cohort study*. Int J Infect Dis, 2020.
56. Lescure, F.X., et al., *Clinical and virological data of the first cases of COVID-19 in Europe: a case series*. Lancet Infect Dis, 2020.
57. Ling, Y., et al., *Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients*. Chin Med J (Engl), 2020. **133**(9): p. 1039-1043.
58. Pan, Y., et al., *Viral load of SARS-CoV-2 in clinical samples*. Lancet Infect Dis, 2020. **20**(4): p. 411-412.
59. Xing, Y.H., et al., *Prolonged viral shedding in feces of pediatric patients with coronavirus disease 2019*. J Microbiol Immunol Infect, 2020.
60. Oliver S, O.S.J., Patel M, Patel S, Queen I, Quick N et al, *Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States*. Nat Med, 2020.
61. Jeroen J.A. van Kampen, D.A.M.C.v.d.V., Pieter L.A. Fraaij, Bart L. Haagmans, Mart M. Lamers, Nisreen Okba, Johannes P.C. van den Akker, Henrik Endeman, Diederik A.M.P.J. Gommers, Jan J. Cornelissen, Rogier A.S. Hoek, Menno M. van der Eerden, Dennis A. Hesselink, Herold J. Metselaar, Annelies Verbon, Jurriaan E.M. de Steenwinkel, Georgina I. Aron, Eric C.M. van Gorp, Sander van Boheemen, Jolanda C. Voermans, Charles A.B. Boucher, Richard Molenkamp, Marion P.G. Koopmans, Corine Geurtsvankessel, Annemiek A. van der Eijk, *Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants*. medRxiv preprint, 2020.
62. La Scola, B., et al., *Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards*. Eur J Clin Microbiol Infect Dis, 2020. **39**(6): p. 1059-1061.

63. Perera, R., et al., *SARS-CoV-2 Virus Culture and Subgenomic RNA for Respiratory Specimens from Patients with Mild Coronavirus Disease*. *Emerg Infect Dis*, 2020. **26**(11).
64. Singanayagam A, P.M., Charlett A, Lopez Bernal J, Saliba V, Ellis J, Ladhani S, Zambon M, Gopal R, *Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020*. *Eurosurveillance*, 2020. **25**(32).
65. *Scientific brief: Criteria to release COVID-19 patients from isolation*. World Health Organization 2020; Available from: <https://apps.who.int/iris/handle/10665/332451>.
66. Yuan, J., et al., *PCR Assays Turned Positive in 25 Discharged COVID-19 Patients*. *Clin Infect Dis*, 2020.
67. Tang, X., et al., *Positive RT-PCR tests among discharged COVID-19 patients in Shenzhen, China*. *Infect Control Hosp Epidemiol*, 2020: p. 1-2.
68. Ma, H., et al., *A single-center, retrospective study of COVID-19 features in children: a descriptive investigation*. *BMC Med*, 2020. **18**(1): p. 123.
69. Xiao, A.T., Y.X. Tong, and S. Zhang, *False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence*. *J Med Virol*, 2020.
70. Liu, R., et al., *Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020*. *Clin Chim Acta*, 2020. **505**: p. 172-175.
71. Winichakoon, P., et al., *Negative Nasopharyngeal and Oropharyngeal Swabs Do Not Rule Out COVID-19*. *J Clin Microbiol*, 2020. **58**(5).
72. Li, Y., et al., *Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19*. *J Med Virol*, 2020.
73. Lee, T.H., et al., *Testing for SARS-CoV-2: Can We Stop at Two?* *Clin Infect Dis*, 2020.
74. Kucirka, L.M., et al., *Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure*. *Ann Intern Med*, 2020.
75. Wang, W., et al., *Detection of SARS-CoV-2 in Different Types of Clinical Specimens*. *JAMA*, 2020.
76. Huang, Y., et al., *SARS-CoV-2 Viral Load in Clinical Samples from Critically Ill Patients*. *Am J Respir Crit Care Med*, 2020. **201**(11): p. 1435-1438.
77. Wong, M.C., et al., *Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: A meta-analysis*. *J Infect*, 2020. **81**(2): p. e31-e38.
78. Chen, W., et al., *Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity*. *Emerg Microbes Infect*, 2020. **9**(1): p. 469-473.
79. Chen, X., et al., *Detectable serum SARS-CoV-2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients*. *Clin Infect Dis*, 2020.
80. Corman, V.M., et al., *SARS-CoV-2 asymptomatic and symptomatic patients and risk for transfusion transmission*. *Transfusion*, 2020.
81. Zhang, W., et al., *Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes*. *Emerg Microbes Infect*, 2020. **9**(1): p. 386-389.
82. Williams, E., et al., *Saliva as a non-invasive specimen for detection of SARS-CoV-2*. *J Clin Microbiol*, 2020.
83. Pasomsub, E., et al., *Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease-2019 (COVID-19): a cross-sectional study*. *Clin Microbiol Infect*, 2020.
84. Yang, J.R., et al., *Persistent viral RNA positivity during the recovery period of a patient with SARS-CoV-2 infection*. *J Med Virol*, 2020.
85. Guo, W.L., et al., *Effect of throat washings on detection of 2019 novel coronavirus*. *Clin Infect Dis*, 2020.
86. Lai, C.K.C., et al., *Prospective study comparing deep-throat saliva with other respiratory tract specimens in the diagnosis of novel coronavirus disease (COVID-19)*. *J Infect Dis*, 2020.
87. Azzi, L., et al., *Saliva is a reliable tool to detect SARS-CoV-2*. *Journal of Infection*, 2020. **81**.
88. McCormick-Baw, C., et al., *Saliva as an Alternate Specimen Source for Detection of SARS-CoV-2 in Symptomatic Patients Using Cepheid Xpert Xpress SARS-CoV-2*. *J Clin Microbiol*, 2020.
89. Colavita, F., et al., *SARS-CoV-2 Isolation From Ocular Secretions of a Patient With COVID-19 in Italy With Prolonged Viral RNA Detection*. *Ann Intern Med*, 2020.
90. Xia, J., et al., *Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection*. *J Med Virol*, 2020.
91. Wu, P., et al., *Characteristics of Ocular Findings of Patients With Coronavirus Disease 2019 (COVID-19) in Hubei Province, China*. *JAMA Ophthalmol*, 2020.
92. Zhou, Y., et al., *Ocular Findings and Proportion with Conjunctival SARS-COV-2 in COVID-19 Patients*. *Ophthalmology*, 2020.
93. Zhang, X., et al., *The evidence of SARS-CoV-2 infection on ocular surface*. *Ocul Surf*, 2020.
94. Cai, J., et al., *A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features*. *Clin Infect Dis*, 2020.

95. Nomoto, H., et al., *Cautious handling of urine from moderate to severe COVID-19 patients*. Am J Infect Control, 2020. **48**(8): p. 969-971.
96. Li, D., et al., *Clinical Characteristics and Results of Semen Tests Among Men With Coronavirus Disease 2019*. JAMA Netw Open, 2020. **3**(5): p. e208292.
97. Paniz-Mondolfi, A., et al., *Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)*. J Med Virol, 2020. **92**(7): p. 699-702.
98. Moriguchi, T., et al., *A first case of meningitis/encephalitis associated with SARS-Coronavirus-2*. Int J Infect Dis, 2020. **94**: p. 55-58.
99. *World Health Organization. Clinical management of COVID-19 (Interim Guidance)* World Health Organization 2020 27 May 2020; Available from: <https://apps.who.int/iris/handle/10665/332196>.
100. Bordi, L., et al., *Differential diagnosis of illness in patients under investigation for the novel coronavirus (SARS-CoV-2), Italy, February 2020*. Euro Surveill, 2020. **25**(8).
101. Wu, D., et al., *To alert coinfection of COVID-19 and dengue virus in developing countries in the dengue-endemic area*. Infect Control Hosp Epidemiol, 2020: p. 1.
102. Rodriguez, J.A., et al., *Co-Infection with SARS-COV-2 and Parainfluenza in a young adult patient with pneumonia: Case Report*. IDCases, 2020. **20**: p. e00762.
103. Rawson, T.M., et al., *Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing*. Clin Infect Dis, 2020.
104. Nowak, M.D., et al., *Co-infection in SARS-CoV-2 infected Patients: Where Are Influenza Virus and Rhinovirus/Enterovirus?* J Med Virol, 2020.
105. Wu, D., et al., *Coinfection of Influenza Virus and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2)*. Pediatr Infect Dis J, 2020. **39**(6): p. e79.
106. Wu, X., et al., *Co-infection with SARS-CoV-2 and Influenza A Virus in Patient with Pneumonia, China*. Emerg Infect Dis, 2020. **26**(6): p. 1324-1326.
107. Khodamoradi, Z., M. Moghadami, and M. Lotfi, *Co-infection of Coronavirus Disease 2019 and Influenza A: A Report from Iran*. Arch Iran Med, 2020. **23**(4): p. 239-243.
108. Azekawa, S., et al., *Co-infection with SARS-CoV-2 and influenza A virus*. IDCases, 2020. **20**: p. e00775.
109. Koehler, P., et al., *COVID-19 associated pulmonary aspergillosis*. Mycoses, 2020.
110. Yan, G., et al., *Covert COVID-19 and false-positive dengue serology in Singapore*. Lancet Infect Dis, 2020. **20**(5): p. 536.
111. Lustig, Y., et al., *Potential antigenic cross-reactivity between SARS-CoV-2 and Dengue viruses*. Clin Infect Dis, 2020.
112. Hammitt, L.L., et al., *Added value of an oropharyngeal swab in detection of viruses in children hospitalized with lower respiratory tract infection*. J Clin Microbiol, 2011. **49**(6): p. 2318-20.
113. Ek, P., et al., *A combination of naso- and oropharyngeal swabs improves the diagnostic yield of respiratory viruses in adult emergency department patients*. Infect Dis (Lond), 2019. **51**(4): p. 241-248.
114. Sutjipto s, H.L., Yant TJ, Mendis SM, Abdad MY, Marimuthu K, Ng OT, Lin C, Chan M et al., *The effect of sample site, illness duration and the presence of pneumonia on the detection of SARS-CoV-2 by real-time reverse-transcription PCR*. Open Forum Infectious Diseases, 2020.
115. Lieberman, D., et al., *Pooled nasopharyngeal and oropharyngeal samples for the identification of respiratory viruses in adults*. Eur J Clin Microbiol Infect Dis, 2010. **29**(6): p. 733-5.
116. Vlek, A.L.M., et al., *Combined throat/nasal swab sampling for SARS-CoV-2 is equivalent to nasopharyngeal sampling*. Eur J Clin Microbiol Infect Dis, 2020.
117. LeBlanc, J.J., et al., *A combined oropharyngeal/nares swab is a suitable alternative to nasopharyngeal swabs for the detection of SARS-CoV-2*. J Clin Virol, 2020. **128**: p. 104442.
118. Pinninti, S., et al., *Comparing Nasopharyngeal and Mid-Turbinate Nasal Swab Testing for the Identification of SARS-CoV-2*. Clin Infect Dis, 2020.
119. Palmas, G., et al., *Nasal Swab as Preferred Clinical Specimen for COVID-19 Testing in Children*. Pediatr Infect Dis J, 2020. **39**(9): p. e267-e270.
120. Tu, Y.P., et al., *Swabs Collected by Patients or Health Care Workers for SARS-CoV-2 Testing*. N Engl J Med, 2020. **383**(5): p. 494-496.
121. Altamirano, J., et al., *Assessment of Sensitivity and Specificity of Patient-Collected Lower Nasal Specimens for Sudden Acute Respiratory Syndrome Coronavirus 2 Testing*. JAMA Netw Open, 2020. **3**(6): p. e2012005.
122. Hamid, H., et al., *COVID-19 Pandemic and Role of Human Saliva as a Testing Biofluid in Point-of-Care Technology*. Eur J Dent, 2020.
123. Alizargar, J., et al., *Saliva samples as an alternative for novel coronavirus (COVID-19) diagnosis*. J Formos Med Assoc, 2020.
124. Ceron, J.J., et al., *Use of Saliva for Diagnosis and Monitoring the SARS-CoV-2: A General Perspective*. J Clin Med, 2020. **9**(5).

125. Chen L, Z.J., Peng J, Li X, Deng X, Shen Z, Guo F, Zhang Q, Zhang Q, Jin Y, Wang L, Wang S *Detection of 2019-nCoV in Saliva and Characterization of Oral Symptoms in COVID-19 Patients*. SSRN, 2020.
126. Ng, S.C., F.K.L. Chan, and P.K.S. Chan, *Screening FMT donors during the COVID-19 pandemic: a protocol for stool SARS-CoV-2 viral quantification*. *Lancet Gastroenterol Hepatol*, 2020. **5**(7): p. 642-643.
127. Tang, J.W., et al., *Quantitative temporal-spatial distribution of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in post-mortem tissues*. *J Med Virol*, 2007. **79**(9): p. 1245-53.
128. Nicholls, J.M., et al., *Lung pathology of fatal severe acute respiratory syndrome*. *Lancet*, 2003. **361**(9371): p. 1773-8.
129. Pomara, C., G. Li Volti, and F. Cappello, *COVID-19 Deaths: Are We Sure It Is Pneumonia? Please, Autopsy, Autopsy, Autopsy!* *J Clin Med*, 2020. **9**(5).
130. Salerno, M., et al., *No Autopsies on COVID-19 Deaths: A Missed Opportunity and the Lockdown of Science*. *J Clin Med*, 2020. **9**(5).
131. Hanley, B., et al., *Autopsy in suspected COVID-19 cases*. *J Clin Pathol*, 2020. **73**(5): p. 239-242.
132. Basso, C., et al., *Feasibility of postmortem examination in the era of COVID-19 pandemic: the experience of a Northeast Italy University Hospital*. *Virchows Arch*, 2020.
133. Tian, S., et al., *Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies*. *Mod Pathol*, 2020. **33**(6): p. 1007-1014.
134. *WHO Guidance on regulations for the Transport of Infectious Substances 2019-2020*. World Health Organization 2019; Available from: <https://apps.who.int/iris/handle/10665/325884>.
135. *Guidance for laboratories shipping specimens to WHO reference laboratories that provide confirmatory testing for COVID-19 virus*. World Health Organization 2020; Available from: <https://apps.who.int/iris/handle/10665/331639>.
136. *WHO laboratory biosafety manual, third edition* World Health Organization 2004; Available from: <https://apps.who.int/iris/handle/10665/42981>.
137. Corman, V.M., et al., *Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR*. *Euro Surveill*, 2020. **25**(3).
138. LeBlanc, J.J., et al., *Real-time PCR-based SARS-CoV-2 detection in Canadian Laboratories*. *J Clin Virol*, 2020: p. 104433.
139. FIND. *SARS-COV-2 molecular assay evaluation results 2020*; Available from: <https://www.finddx.org/covid-19/sarscov2-eval-molecular/molecular-eval-results/>.
140. Uhteg, K., et al., *Comparing the analytical performance of three SARS-CoV-2 molecular diagnostic assays*. *J Clin Virol*, 2020. **127**: p. 104384.
141. van Kasteren, P.B., et al., *Comparison of seven commercial RT-PCR diagnostic kits for COVID-19*. *J Clin Virol*, 2020. **128**: p. 104412.
142. Lowe, C.F., et al., *Detection of low levels of SARS-CoV-2 RNA from nasopharyngeal swabs using three commercial molecular assays*. *J Clin Virol*, 2020. **128**: p. 104387.
143. Igloi, Z., et al., *Comparison of commercial realtime reverse transcription PCR assays for the detection of SARS-CoV-2*. *J Clin Virol*, 2020. **129**: p. 104510.
144. Dinnes J, D.J., Adriano A, Berhane S, Davenport C, Dittrich S, Emperador D, Takwoingi Y, Cunningham J, Beese S, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Taylor-Phillips S, Hooft L, Leeflang MMG, Spijker R, Van den Bruel A. , *Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection*. *Cochrane Database of Systematic Reviews* 2020(8).
145. Carter, L.J., et al., *Assay Techniques and Test Development for COVID-19 Diagnosis*. *ACS Cent Sci*, 2020. **6**(5): p. 591-605.
146. *Rapid HTA of Alternative Diagnostic Technologies for the Detection of SARS-CoV-2*. 2020; Available from: https://www.hiqa.ie/sites/default/files/2020-05/Rapid_HTA_COVID-19_tests.pdf.
147. Esbin, M.N., et al., *Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection*. *RNA*, 2020. **26**(7): p. 771-783.
148. Fomsgaard, A.S. and M.W. Rosenstjerne, *An alternative workflow for molecular detection of SARS-CoV-2 - escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020*. *Euro Surveill*, 2020. **25**(14).
149. Alcoba-Florez, J., et al., *Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples*. *Int J Infect Dis*, 2020. **97**: p. 66-68.
150. Chen, H., et al., *Influence of Different Inactivation Methods on Severe Acute Respiratory Syndrome Coronavirus 2 RNA Copy Number*. *J Clin Microbiol*, 2020. **58**(8).
151. Bentley, D.R., et al., *Accurate whole human genome sequencing using reversible terminator chemistry*. *Nature*, 2008. **456**(7218): p. 53-59.
152. Chu, A.W., et al., *Evaluation of simple nucleic acid extraction methods for the detection of SARS-CoV-2 in nasopharyngeal and saliva specimens during global shortage of extraction kits*. *J Clin Virol*, 2020. **129**: p. 104519.

153. Hasan, M.R., et al., *Detection of SARS-CoV-2 RNA by direct RT-qPCR on nasopharyngeal specimens without extraction of viral RNA*. PLoS One, 2020. **15**(7): p. e0236564.
154. Mancini, F., et al., *Laboratory management for SARS-CoV-2 detection: a user-friendly combination of the heat treatment approach and rt-Real-time PCR testing*. Emerg Microbes Infect, 2020. **9**(1): p. 1393-1396.
155. Yelin, I., et al., *Evaluation of COVID-19 RT-qPCR test in multi-sample pools*. Clin Infect Dis, 2020.
156. Mallapaty, S., *The mathematical strategy that could transform coronavirus testing*. Nature, 2020.
157. Williams, B.G., *Optimal pooling strategies for laboratory testing*. arXiv, 2010. **1007.4903**: p. 1-3.
158. Khodare, A., et al., *Optimal size of sample pooling for RNA pool testing: An avant-garde for scaling up severe acute respiratory syndrome coronavirus-2 testing*. Indian J Med Microbiol, 2020. **38**(1): p. 18-23.
159. Abdalhamid, B., et al., *Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources*. Am J Clin Pathol, 2020. **153**(6): p. 715-718.
160. Aragon-Caqueo, D., J. Fernandez-Salinas, and D. Laroze, *Optimization of group size in pool testing strategy for SARS-CoV-2: A simple mathematical model*. J Med Virol, 2020.
161. Pilcher, C.D., D. Westreich, and M.G. Hudgens, *Group Testing for Sars-Cov-2 to Enable Rapid Scale-Up of Testing and Real-Time Surveillance of Incidence*. J Infect Dis, 2020.
162. Ben-Ami, R., et al., *Large-scale implementation of pooled RNA extraction and RT-PCR for SARS-CoV-2 detection*. Clin Microbiol Infect, 2020.
163. Lambert-Niclot, S., et al., *Evaluation of a rapid diagnostic assay for detection of SARS CoV-2 antigen in nasopharyngeal swab*. J Clin Microbiol, 2020.
164. Mertens, P., et al., *Development and Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context*. Front Med (Lausanne), 2020. **7**: p. 225.
165. Porte, L., et al., *Evaluation of novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples*. Int J Infect Dis, 2020.
166. Zhao, J., et al., *Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019*. Clin Infect Dis, 2020.
167. Okba, N.M.A., et al., *Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients*. Emerg Infect Dis, 2020. **26**(7).
168. Lou, B., et al., *Serology characteristics of SARS-CoV-2 infection since exposure and post symptom onset*. Eur Respir J, 2020.
169. Zhou, P., et al., *A pneumonia outbreak associated with a new coronavirus of probable bat origin*. Nature, 2020. **579**(7798): p. 270-273.
170. Dutch National Taksforce serology, R.C., Murk J, van den Beld M, Reimerink J, Kluytmans J, Wegdam M, Zaaijer H, van Loo I, Geurts van Kessel C, Koopmans M. . *Report Status of the validation of point-of-care serology tests for SARS-CoV-2 diagnostics: considerations for use*. 15 July 2020; Available from: https://www.nvmm.nl/media/3666/status-validation-poc-ab-tests_20200715_final.pdf.
171. Dutch National Taksforce serology, R.C., Murk J, van den Beld M, Reimerink J, Kluytmans J, Wegdam M, Zaaijer H, van Loo I, Geurts van Kessel C, Koopmans M. *Report Status of the validation of ELISA and auto-analyser antibody tests for SARS-CoV-2 diagnostics: consideratoins for use*. 15 July 2020; Available from: https://www.nvmm.nl/media/3667/status-validation-elisa-and-auto-analysers_20200715_final.pdf.
172. Fafi-Kremer, S., et al., *Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France*. EBioMedicine, 2020: p. 102915.
173. Deeks J, D.J., Takwoingi Y, Davenport C, Spijker R, Taylor-Philips et al. , *Antibody tests for identification of current and past infection with SARS-CoV-2*. Cochrane Library, 2020.
174. Jeffrey Seow, C.G., Blair Merrick, Sam Acors, Kathryn J.A. Steel and K.J.D. 10 Malim1, *Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection*. medrxiv 2020.
175. Caini, S., et al., *Meta-analysis of diagnostic performance of serological tests for SARS-CoV-2 antibodies up to 25 April 2020 and public health implications*. Euro Surveill, 2020. **25**(23).
176. Lisboa Bastos, M., et al., *Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis*. BMJ, 2020. **370**: p. m2516.
177. GeurtsvanKessel, C.H., et al., *An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment*. Nat Commun, 2020. **11**(1): p. 3436.
178. Che, X.Y., et al., *Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43*. J Infect Dis, 2005. **191**(12): p. 2033-7.
179. Meyer, B., C. Drosten, and M.A. Muller, *Serological assays for emerging coronaviruses: challenges and pitfalls*. Virus Res, 2014. **194**: p. 175-83.
180. Gorse, G.J., M.M. Donovan, and G.B. Patel, *Antibodies to coronaviruses are higher in older compared with younger adults and binding antibodies are more sensitive than neutralizing antibodies in identifying coronavirus-associated illnesses*. J Med Virol, 2020. **92**(5): p. 512-517.

181. Theel E, F.L., Palavecino, E et al. , *Verification procedure for commercial serologic tests with Emergency Use Authorization for detection of antibodies to SARS-CoV-2*. American society for microbiology, 2020.
182. To, K.K., et al., *COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing*. Clin Infect Dis, 2020.
183. Hin Chu, J.F.-W.C., Terrence Tsz-Tai Yuen, Huiping Shuai, Shuofeng Yuan, Yixin Wang, et al, *Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study*. The Lancet Microbe, 2020. **Volume 1**(ISSUE 1): p. e14-e23.
184. Mogling, R., et al., *Delayed Laboratory Response to COVID-19 Caused by Molecular Diagnostic Contamination*. Emerg Infect Dis, 2020. **26**(8).
185. *WHO reference laboratories providing confirmatory testing for COVID-19*. World Health Organization 2020 Available from: <https://www.who.int/publications/m/item/who-reference-laboratories-providing-confirmatory-testing-for-covid-19>.
186. *World Health Organization. International Health Regulations (2005), third edition*. . World Health Organization 2016; Available from: <http://www.who.int/ihr/publications/9789241580496/en/>.
187. Daniel B Larremore, B.W., Evan Lester, Soraya Shehata, James M Burke, James A Hay, Milind Tambe, Michael J Mina, Roy Parker, *Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance*. Medrxiv preprint, 2020.
188. Kretzschmar, M.E., et al., *Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study*. Lancet Public Health, 2020. **5**(8): p. e452-e459.
189. *Laboratory testing for Middle East Respiratory Syndrome coronavirus, interim guidance (revised)*. World Health Organization, 2019.
190. *WHO Global Influenza Surveillance Network Manual for the laboratory diagnosis and virological surveillance of influenza*. World Health Organization, 2011.
191. *WHO Recommended Surveillance Standards WHO/CDS/CSR/ISR/99.2*. World Health Organization, 1999.
192. *Guideline for the collection of clinical specimens during field investigation of outbreaks WHO/CDS/CSR/EDC/200.4* World Health Organization, 2000.
193. *Managing epidemics, key facts about major deadly diseases*. . World Health Organization, 2018.
194. *Protocol to investigate non-seasonal influenza and other emerging acute respiratory diseases*. World Health Organization, 2018.
195. Rodino, K.G., et al., *Evaluation of Saline, Phosphate-Buffered Saline, and Minimum Essential Medium as Potential Alternatives to Viral Transport Media for SARS-CoV-2 Testing*. J Clin Microbiol, 2020. **58**(6).
196. Poon, P.c.L., *Evaluation of swabs, transport media and specimen transport conditions for the detection of COVID-19 virus by RT-PCR*. University of Hong Kong, 2020.
197. Rogers, A.A., et al., *Evaluation of Transport Media and Specimen Transport Conditions for the Detection of SARS-CoV-2 Using Real Time Reverse Transcription PCR*. J Clin Microbiol, 2020.
198. Radbel, J., et al., *Detection of SARS-CoV-2 is comparable in clinical samples preserved in saline or viral transport media*. J Mol Diagn, 2020.

WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance will expire 1 year after the date of publication.

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WHO reference number: [WHO/2019-nCoV-19/laboratory/2020.6](https://www.who.int/publications/m/item/who-reference-laboratories-providing-confirmatory-testing-for-covid-19)

Annex 1: Specimen collection and storage

Specimen type	Collection materials	Recommended temperature for storage and/or shipment to the laboratory and until testing (from date of specimen collection) #
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs with VTM *	2-8 °C if ≤12 days* -70 °C (dry ice) if > 12 days
Bronchoalveolar lavage	Sterile container with viral transport medium **	2-8 °C if ≤ 2 days -70 °C (dry ice) if > 2 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container with viral transport medium**	2-8 °C if ≤2 days -70 °C (dry ice) if > 2 days
Sputum	Sterile container	2-8 °C if ≤ 2 days -70 °C (dry ice) if > 2 days
Tissue from biopsy or autopsy including from lung	Sterile container with saline or VTM	2-8 °C if ≤ 24 hours -70 °C (dry ice) if > 24 hours
Serum	Serum separator tubes (adults: collect 3-5 ml whole blood)	2-8 °C if ≤ 5 days -70 °C (dry ice) if > 5 days
Whole blood	Collection tube	2-8 °C if ≤5 days -70 °C (dry ice) if > 5 days
Stool	Stool container	2-8 °C if ≤5 days -70 °C (dry ice) if > 5 days

Avoid repeated freezing and thawing of specimens. If no access to -70 °C consider storing at -20 °C.

* For transport of specimens for viral detection, use preferentially viral transport medium (VTM) containing antifungal and antibiotic supplements. If VTM is not available, other solutions may be used after validation. Such solution may include phosphate buffered saline (PBS), 0.9% sterile saline, minimum essential medium (with storage at +4C up to 7 to 14 days) [195-197]. In case other viruses such as influenza should also be tested, do not store samples for more than 5 days at 4-8 degrees but -70 °C or dry ice [194].

** If VTM is not available, sterile saline may be used [198]. Duration of specimen storage at 2-8 °C may be different from what is indicated above.

Apart from specific collection materials indicated in the table, ensure that other materials and equipment are available: e.g. transport containers and specimen collection bags and packaging, coolers, and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, materials for decontamination of surfaces, etc.

Annex 2: laboratory request form

SARS-CoV-2 LABORATORY TEST REQUEST FORM¹

Submitter information			
NAME OF SUBMITTING HOSPITAL, LABORATORY, or OTHER FACILITY*			
Physician			
Address			
Phone number			
Case definition ² :	<input type="checkbox"/> Suspected case <input type="checkbox"/> Probable case <input type="checkbox"/> Other:		
Patient info			
First name		Last name	
Patient ID number		Date of Birth	Age:
Address		Sex	<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Unknown
Phone number			
Specimen information			
Type	<input type="checkbox"/> Nasopharyngeal and oropharyngeal swab <input type="checkbox"/> Bronchoalveolar lavage <input type="checkbox"/> Endotracheal aspirate <input type="checkbox"/> Nasopharyngeal aspirate <input type="checkbox"/> Nasal wash <input type="checkbox"/> Sputum <input type="checkbox"/> Lung tissue <input type="checkbox"/> Serum <input type="checkbox"/> Whole blood <input type="checkbox"/> Stool <input type="checkbox"/> Other:		
All specimens collected should be regarded as potentially infectious and you <u>must contact</u> the reference laboratory <u>before</u> sending specimens to them. All specimens must be sent in accordance with category B transport requirements.			
Please tick the box if your clinical specimen is post mortem <input type="checkbox"/>			
Date of collection		Time of collection	
Priority status			
Clinical details			
Date of symptom onset:			
Has the patient had a recent history of travelling to an affected area?	<input type="checkbox"/> Yes	Country	
	<input type="checkbox"/> No	Return date	
Has the patient had contact with a confirmed case?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown <input type="checkbox"/> Other exposure:		
Additional Comments (e.g. antimicrobial treatment, immunosuppressants)			

¹ Form in accordance with ISO 15189:2012 requirements² [Public health surveillance for COVID-19: interim guidance](#)

Annex 3: Considerations when selecting the optimal NAAT for the use setting

Aspect	Considerations
Manufacturing quality	CE-IVD, WHO EUL, PQ, EU-FDA or other approval. Independent validation data. Manufacture under ISO.
Targets	Number of targets, specificity for SARS-CoV-2 or other sarbecoviruses.
Controls	For manual NAAT testing, a positive template control (PTC) and at least one negative template control (NTC) should be included. Use of an extraction control and an internal human housekeeping gene specimen adequacy control is also recommended.
Instrumentation	Is the assay compatible with available systems in the laboratory or country? Ease of use and operational utility. Opportunity to multiplex with other respiratory pathogens. Costing of platform and maintenance. Ease of access to maintenance provider/troubleshooting.
Workflow	Can the kit be implemented in the existing workflow of the laboratory, while assuring minimal disruption on other diagnostics?
Ease of use	Complexity of assay. Number of steps. Required training and staff.
Storage and shipment requirement	Many kits require cold chain conditions during shipment and storage, in some circumstances this might pose a challenge. Some kits contain lyophilized enzymes that do not require the kit to be shipped and sometimes stored cold. Shelf life: To be prepared for periods of intense testing stocks might be needed, a longer shelf life is needed to ensure adequate use of resources.
Training needs and access	Instructions for use (IFU) available, training available by company or others, troubleshooting options provided and accessible help line in local language.
Need for ancillary reagents	Complete kit for sampling/extraction/amplification or the PCR kit requires additional reagents or tools. Compatibility with laboratories' extraction method. Compatibility with procurable polymerases if needed. Special equipment needed (e.g. calibration panel before running the test, extraction platforms, heat block, vortex, magnetic stand or centrifuge).
Continuity of supply	Long term supply agreement. Secured routes of delivery if lockdowns occur. Assay and ancillary reagents costs.

Annex 4: Suggestions for checklist to reduce possible cases of false positive rRT-PCR results and handling of equivocal results

Laboratories should have a standard operating procedure in place to reduce the possible false positive rRT-PCR results and how to handle equivocal results. This checklist provides the laboratories with suggestions and considerations. The checklist is formulated for manual rRT-PCRs but many aspects can also be used for other NAATs.

CLERICAL

- Eliminate or reduce transcription
- If transcribe, method of checking
- Sorting, aliquoting and labelling
- Double identifiers
- Entering results

CROSS CONTAMINATION

- Preparation area
- Manipulation of tubes
- Aerosol generation
- Nucleic acid concentration and extraction setup
- PCR format and steps
- Check other positives in same run
- Environmental
- Contaminated reagents
- Disposal

EQUIPMENT and TEST KITS

- Calibration method
- Equipment validated for test kit
- Assess new equipment for contamination risk

PRACTICE

- For mass screening, separate high-prevalence from low-prevalence groups.
- Visual inspection of run
- Analytical – examination of raw data
- Extend run when necessary for late Ct

EQUIVOCAL RESULTS

- Follow manufacturer's instructions
- Laboratory policy for equivocal results
- Any additional laboratory criteria for equivocal category
- Communication of interpretation to users
- Criteria for repeat testing, if any
- Use of alternative test or PCR target
- Communication with clinical and public health staff