Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases.

Interim guidance
2 March 2020

World Health Organization

1. Introduction

Several coronaviruses can infect humans, the globally endemic human coronaviruses HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 that tend to cause mild respiratory disease, and the zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) that have a higher case fatality rate. In December 2019, a cluster of patients with a novel coronavirus was identified in Wuhan, China (1). Initially tentatively named 2019 novel coronavirus (2019-nCoV), the virus has now been named SARS-CoV-2 by the International Committee of Taxonomy of Viruses (ICTV) (2). This virus can cause the disease named coronavirus disease 2019 (COVID-19). WHO refers to the virus as COVID-19 virus in its current documentation.

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in COVID-19 virus laboratory testing of patients.

Existing WHO documents have been consulted for drafting this interim guidance, including the interim guidance on laboratory testing for MERS (3-9). Information on human infection with the COVID-19 virus is evolving and WHO continues to monitor developments and revise recommendations as necessary. Feedback is welcome and can be sent to WHElab@who.int.

2. Laboratory testing guiding principles for patients who meet the suspect case definition

The decision to test should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection. PCR testing of asymptomatic or mildly symptomatic contacts can be considered in the assessment of individuals who have had contact with a COVID-19 case. Screening protocols should be adapted to the local situation. The case definitions are being regularly reviewed and updated as new information becomes available. For the WHO suspect case definition see: Global Surveillance for human infection with coronavirus disease (COVID-2019) (10).

Rapid collection and testing of appropriate specimens from patients meeting the suspect case definition for COVID-19 is a priority for clinical management and outbreak control and should be guided by a laboratory expert. Suspect cases should be screened for the virus with **nucleic acid amplification tests (NAAT), such as RT-PCR.**

If testing for COVID-19 is not yet available nationally, specimens should be referred. A list of WHO reference laboratories providing confirmatory testing for COVID-19 and shipment instructions are available in Section 4 of the following webpage: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.

If case management requires, patients should be tested for other respiratory pathogens using routine laboratory procedures, as recommended in local management guidelines for community-acquired pneumonia. Additional testing should not delay testing for COVID-19. As co-infections can occur, all patients that meet the suspect case definition should be tested for COVID-19 virus regardless of whether another respiratory pathogen is found.

In an early study in Wuhan, the mean incubation period for COVID-19 was 5.2 days among 425 cases, though it varies widely between individuals (11,12,13). Virus shedding patterns are not yet well understood and further investigations are needed to better understand the timing, compartmentalization and quantity of viral shedding to inform optimal specimen collection. Though respiratory samples have the greatest yield, the virus can be detected in other specimens, including stool and blood (14,15,16). Local guidelines should be followed regarding patient or guardian's informed consent for specimen collection, testing and potentially future research.

3. Specimen collection and shipment

Safety procedures during specimen collection
Ensure that adequate SOPs are in use and that staff are trained for appropriate specimen collection, storage, packaging and transport. All specimens collected for laboratory investigations should be regarded as potentially infectious.

Ensure that health care workers who collect specimens adhere rigorously to infection prevention and control guidelines. Specific WHO interim guidance has been published: "Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected, interim guidance, January 2020" (17) and "WHO interim guidance for laboratory biosafety related to 2019-nCoV" (18).

Box 1. Biosafety practices in the laboratory

Testing on clinical specimens from patients meeting the suspect case definition 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. There is still limited information on the risk posed by COVID-19, but all procedures should be undertaken based on a risk assessment. Specimen handling for molecular testing would require BSL-2 or equivalent facilities. Attempts to culture the virus require BSL-3 facilities at minimum.

For more information related to COVID-19 risk assessment, see specific interim guidance document: 'WHO interim guidance for laboratory biosafety related to 2019-nCoV' (18). Samples that are potentially infectious materials (PIM) for polio need to be handled and stored as described in WHO document "Guidance to minimize risks for facilities collecting, handling or storing materials potentially infectious for polioviruses (PIM Guidance)" (23). For general laboratory biosafety guidelines, see the WHO Laboratory Biosafety Manual, 3rd edition before the 4th edition is released.

Specimens to be collected
At minimum, **respiratory material** should be collected:

- **upper respiratory specimens:** nasopharyngeal and oropharyngeal swab or wash in ambulatory patients
- and/or **lower respiratory specimens:** sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. (Note high risk of aerosolization; adhere strictly to infection prevention and control procedures).

Additional clinical specimens may be collected as COVID-19 virus has been detected in blood and stool, as had the coronaviruses responsible for SARS and MERS (14,16,19-21). The duration and frequency of shedding of COVID-19 virus in stool and potentially in urine is unknown. In case of patients who are deceased, consider autopsy material including lung tissue. In surviving patients, paired serum (acute and convalescent) can be useful to retrospectively define cases as serological assays become available.

Further recommendations on materials to collect, including the testing of asymptomatic individuals, can be found in Table 1.

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 is essential. Specimens which can be delivered promptly to the laboratory can be stored and shipped at 2-8°C. When there is likely to be a delay in specimens reaching the laboratory, the use of viral transport medium is strongly recommended. Specimens may be frozen to - 20°C or ideally -70°C and shipped on dry ice if further delays are expected (see Table 2). It is important to avoid repeated freezing and thawing of specimens.

Transport of specimens within national borders should comply with applicable national regulations. International transport of potentially COVID-19 virus containing samples should follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used. More information may be found in the "WHO Guidance on regulations for the Transport of

Infectious Substances 2019-2020"(22) and "WHO interim guidance for laboratory biosafety related to 2019-nCoV"(18).

Ensure good communication with the laboratory and provide needed information
Alerting the laboratory before sending specimens encourages proper and timely processing of samples and timely reporting. Specimens should be correctly labelled and accompanied by a diagnostic request form (template provided in Annex I).

4. Laboratory testing for COVID-19 virus

Laboratories undertaking testing for COVID-19 virus should adhere strictly to appropriate biosafety practices.

Nucleic acid amplification tests (NAAT) for COVID-19 virus
Routine confirmation of cases of COVID-19 is based on detection of unique sequences of virus RNA by NAAT such as real-time reverse-transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary. The viral genes targeted so far include the N, E, S and RdRP genes. Examples of protocols used may be found here. RNA extraction should be done in a biosafety cabinet in a BSL-2 or equivalent facility. Heat treatment of samples prior to RNA extraction is not recommended.

Laboratory confirmation of cases by NAAT in areas with no known COVID-19 virus circulation
To consider a case as laboratory-confirmed by NAAT in an area with no COVID-19 virus circulation, one of the following conditions need to be met:

- A positive NAAT result for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (as at present no other SARS-like coronaviruses are circulating in the human population it can be debated whether it has to be COVID-19 or SARS-like coronavirus specific); **OR**
- One positive NAAT result for the presence of betacoronavirus, and COVID-19 virus further identified by sequencing partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used.

When there are discordant results, the patient should be resampled and, if appropriate, sequencing of the virus from the original specimen or of an amplicon generated from an appropriate NAAT assay, different from the NAAT assay initially used, should be obtained to provide a reliable test result. Laboratories are urged to seek confirmation of any surprising results in an international reference laboratory.

Laboratory confirmed case by NAAT in areas with established COVID-19 virus circulation
In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which for example screening by rRT-PCR of a single discriminatory target is considered sufficient.

One or more negative results do not rule out the possibility of COVID-19 virus infection. A number of factors could lead to a negative result in an infected individual, including:

- poor quality of the specimen, containing little patient material (as a control, consider determining whether there is adequate human DNA in the sample by including a human target in the PCR testing)
- the specimen was collected late or very early in the infection
- the specimen was not handled and shipped appropriately
- technical reasons inherent in the test, e.g. virus mutation or PCR inhibition.

If a negative result is obtained from a patient with a high index of suspicion for COVID-19 virus infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from the lower respiratory tract if possible, should be collected and tested.

Each NAAT run should include both external and internal controls, and laboratories are encouraged to participate in external quality assessment schemes when they become available. It is also recommended to laboratories who order their own primers and probes to perform entry testing/validation on functionality and potential contaminants.

Laboratories with limited experience in testing for COVID-19 virus are encouraged to work with laboratories with more experience with this pathogen to have their initial test results confirmed and to improve their own performance.

For laboratories testing for COVID-19 virus in countries where COVID-19 was not previously circulating, WHO advises the confirmation of testing results for:

- the first 5 positive specimens,
- the first 10 negative specimens (collected from patients that fit the case definition)

by referring them to one of the WHO reference laboratories providing confirmatory testing for COVID-19. For national COVID-19 laboratories that require support with specimen shipment to one of the reference laboratories for testing confirmation, a WHO shipment fund is available. Please refer to the WHO website for the most updated list of reference laboratories and shipment instructions.

Serological testing

Serological surveys can aid investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of an outbreak. In cases where NAAT assays are negative and there is a strong epidemiological link to COVID-19 infection, paired serum samples (in the acute and convalescent phase) could support diagnosis once validated serology tests are available. Serum samples can be stored for these purposes.

Cross reactivity to other coronaviruses can be challenging (24) but commercial and non-commercial serological tests are currently under development. Some studies with COVID-19 serological data on clinical samples have been published (25,26).

Viral sequencing

In addition to providing confirmation of the presence of the virus, regular sequencing of a percentage of specimens from clinical cases can be useful to monitor for viral genome mutations that might affect the performance of medical countermeasures, including diagnostic tests. Virus whole genome sequencing can also inform molecular epidemiology studies. Many public-access databases for deposition of genetic sequence data are available, including GISAID, which is intended to protect the rights of the submitting party (27).

Viral culture

Virus isolation is not recommended as a routine diagnostic procedure.

6. Reporting of cases and test results

Laboratories should follow national reporting requirements. In general, all test results, positive or negative, should be immediately reported to national authorities. States Parties to the IHR are reminded of their obligations to share with WHO relevant public health information for events for which they notified WHO, using the decision instrument in Annex 1 of the IHR (2005) (28).

7. Research toward improved detection of COVID-19 virus

Many aspects of the virus and disease are still not understood. A better understanding will be needed to provide improved guidance. For example:

Viral dynamics: optimal timing and type of clinical material to sample for molecular testing

- Dynamic of immunological response
- Disease severity in various populations, e.g. by age.
- The relationship between viral concentration and disease severity
- The duration of shedding, and relation to clinical picture (e.g. clinical recovery occurs with viral clearing, or shedding persists despite clinical improvement)
- Development and validation of useful serological assays
- Comparative studies of available molecular and serological assays
- Optimal percentage of positive cases that requires sequencing to monitor mutations that might affect the performance of molecular tests.

WHO encourages the sharing of data to better understand and thus manage the COVID-19 outbreak, and to develop countermeasures.

Table 1. Specimens to be collected from symptomatic patients and contacts

	Test	Type of sample	Timing
Patient	NAAT	**Lower respiratory tract** - sputum - aspirate - lavage **Upper respiratory tract** - nasopharyngeal and - oropharyngeal swabs - nasopharyngeal wash/nasopharyngeal aspirate **Consider stools, whole blood, urine, and if diseased, material from autopsy**	Collect on presentation. Possibly repeated sampling to monitor clearance. Further research needed to determine effectiveness and reliability of repeated sampling.
Patient	Serology	Serum for serological testing once validated and available	Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 2-4 weeks later (optimal timing for convalescent sample needs to be established).
Contact (in health-care centre associated outbreaks or other settings where contacts have symptoms, or where asymptomatic contacts have had high-intensity contact with a COVID-19 case.	NAAT	Nasopharyngeal and oropharyngeal swabs	Within incubation period of last documented contact.
	Serology	Serum for serological testing once validated and available	Baseline serum taken as early as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last contact (optimal timing for convalescent sample needs to be established).

Table 2. Specimen collection and storage (adapted from ref 6 and ref 29,30)

Specimen type	Collection materials	Storage temperature until testing in-country laboratory	Recommended temperature for shipment according to expected shipment time
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	2-8 °C	2-8 °C if ⩽ 5 days –70 °C (dry ice) if > 5 days
Bronchoalveolar lavage	Sterile container *	2-8 °C	2-8 °C if ⩽ 2 days –70 °C (dry ice) if > 2 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container *	2-8 °C	2-8 °C if ⩽ 2 days –70 °C (dry ice) if > 2 days
Sputum	Sterile container	2-8 °C	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	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	2-8 °C if ⩽ 5 days –70 °C (dry ice) if > 5 days
Whole blood	Collection tube	2-8 °C	2-8 °C if ⩽ 5 days –70 °C (dry ice) if > 5 days
Stool	Stool container	2-8 °C	2-8 °C if ⩽ 5 days –70 °C (dry ice) if > 5 days
Urine	Urine collection container	2-8 °C	2-8 °C if ⩽ 5 days –70 °C (dry ice) if > 5 days

* For transport of samples for viral detection, use viral transport medium (VTM) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens. If VTM is not available sterile saline may be used in place of VTM (in such case, duration of sample storage at 2-8 °C may be different from what is indicated above).

Aside from specific collection materials indicated in the table also assure 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.

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Annex I

COVID-19 virus LABORATORY TEST REQUEST FORM[1]

Submitter information				
NAME OF SUBMITTING HOSPITAL, LABORATORY, or OTHER FACILITY*				
Physician				
Address				
Phone number				
Case definition[2]:	☐ Suspect case ☐ Probable case			
Patient info				
First name		**Last name**		
Patient ID number		**Date of Birth**		**Age:**
Address		**Sex**	☐ Male ☐ Female ☐ Unknown	
Phone number				
Specimen information				
Type	☐ Nasopharyngeal and oropharyngeal swab ☐ Bronchoalveolar lavage ☐ Endotracheal aspirate ☐ Nasopharyngeal aspirate ☐ Nasal wash ☐ Sputum ☐ Lung tissue ☐ Serum ☐ Whole blood ☐ Urine ☐ Stool ☐ Other: ….			
All specimens collected should be regarded as potentially infectious **and you must contact the reference laboratory before sending samples.** All samples must be sent in accordance with category B transport requirements.				
Please tick the box if your clinical sample is post mortem ☐				
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?	☐ Yes ☐ No	**Country**		
		Return date		
Has the patient had contact with a confirmed case?		☐ Yes ☐ No ☐ Unknown ☐ Other exposure:		
Additional Comments				

WHO reference number: WHO/COVID-19/laboratory/2020.4

[1] Form in accordance with ISO 15189:2012 requirements
[2] https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov)