

Laboratory Guidelines for the Detection and Diagnosis of COVID-19 Virus Infection

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Coronaviruses are a group of highly diverse RNA viruses in the *Coronaviridae* family that are divided in 4 genera: alpha, beta, gamma and delta that cause disease varying from mild to severe in human and animals (1-3). There are endemic human coronavirus as alphacoronaviruses 229E and NL63 and betacoronaviruses OC43 and HKU1 that can cause influenza-like illness or pneumonia in humans (1, 3). However, two zoonotic coronaviruses have emerged causing severe disease in humans: Severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002-2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) (1-5).

In January 2020, the etiologic agent responsible for a cluster of severe pneumonia cases in Wuhan, China, was identified as being a novel betacoronavirus, distinct from SARS-CoV and MERS-CoV (6). On 11 February 2020, the International Committee on Taxonomy of Viruses (ICTV) announced that the virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (7) while, on the same day, WHO named the disease as coronavirus disease COVID-19 (8). For communication purposes we will refer the virus as “the virus responsible for COVID-19” or “the COVID-19 virus”. Complete genomic sequences of the COVID-19 virus have been released and different molecular detection protocols developed but not fully validated yet (9). However, given the current circulation of COVID-19 in the Americas region, the Pan American Health Organization / World Health Organization (PAHO/WHO) recommends to Member States to ensure timely identification of suspect cases, collection and shipping of samples to reference laboratories, and implementation of molecular detection protocols, according to the laboratory capacity.

On 19 March 2020, WHO updated its interim guidance on the Laboratory testing for coronavirus disease (COVID-19) in suspected human cases which includes information on specimen collection and shipment, laboratory testing, and reporting of cases and test results (9). WHO also updates COVID-19 suspect case definitions as needed (10).

Sample collection and proper shipment

Sample collection

Samples should be collected by trained personnel and considering all biosafety instructions including the use of personal protective equipment appropriate for standard, contact, and airborne precautions. In particular, personnel should use proper hand hygiene, gown, respirator (N95 or FFP2), eye (goggle) or facial (face shield) protection, and gloves (11).

Respiratory samples

Recommended samples are those from the lower respiratory tract, including sputum, bronchoalveolar lavage and tracheal aspirate (when possible according to medical criteria). However, when collection of a lower respiratory tract sample is not possible, samples from the upper respiratory tract are also useful. In general, the collection of a combined nasopharyngeal swab and oropharyngeal swab is recommended (swabs should be placed and transported in the same tube with viral or universal transport medium) (9).

If sampling of asymptomatic contacts is included in national guidelines, upper respiratory samples are preferred.

Only Dacron or polyester flocculated swabs should be used. Protocols for the in house production of viral transport media are available upon request to PAHO Regional Office. Additionally, sterile saline might be used if transport medium is not available (see below for sample transport considerations).

Sample shipment

Respiratory samples should be kept refrigerated (4-8 °C) and sent to the laboratory where they will be processed within the 24-72 hours of collection. If samples cannot be sent within this period, freezing at -70 °C (or less) is recommended until samples are shipped (ensuring the cold chain is maintained). If swabs were placed in sterile saline instead of viral transport medium, shipment should be expedited.

Shipment of suspected samples to reference laboratories or collaborating centers outside of the country and by air must ensure compliance with all international standards (IATA) for Biological Substances, Category B (12).

Other sample types

The COVID-19 virus as well as SARS-CoV and MERS-CoV, has been detected in other sample types, such as stools and blood (9). However, the viral dynamics in these samples has not been fully characterized. Samples of lung tissue or respiratory tract might also be useful for molecular detection, as long as the appropriate conditions are in place to perform the autopsy, particularly respiratory protection. Acute and convalescent blood samples might be useful as serological tests become available (see below).

Laboratory testing

Biosafety guidelines for the handling of suspected samples in the laboratory have been published elsewhere (12, 13).

Molecular methods

Routine confirmation of COVID-19 cases is based on detection of COVID-19 virus nucleic acid (RNA) by real time RT-PCR assays.

RNA extraction

RNA can be extracted from samples mentioned above using any standard extraction protocols or kits. In general, the sample lysis step in RNA extraction inactivates any live virus. Thus, lysed samples are generally considered non-infectious. The inactivation of COVID-19 virus through sample lysis has been verified for some commercial kits (14).

Sputum samples require liquification prior to molecular extraction (15), while tissue samples require lysis and homogenization.

Molecular detection protocols

WHO has made available several molecular diagnostic protocols (using RT-PCR) on the following link: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>

Please note that neither the names of vendors nor manufacturers included in the protocols are preferred/endorsed by WHO. Also, these protocols have not yet been validated through WHO process.

Through the effort of PAHO Member States, all national laboratories with the capacity to perform molecular tests, including National Influenza Centers (NIC), were trained in the use of the first protocol made available by WHO, developed by the Charité – Universitätsmedizin Berlin Institute of Virology, Berlin, German. The evaluation of the protocol has been published (16) and a work protocol is available on the following link: <https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf>

The protocol is based on the detection of two targets in the virus genome: the E gene as a screening, followed by confirmation of E gene-positives through the detection of the RdRP gene using the P1 and/or the P2 probe. The E assay is specific for all SARS-CoV-related viruses (*ie*, SARS-CoV, COVID-19 virus, and related bat viruses), while the RdRP assay using the P2 probe only detects the COVID-19 virus. Specific reagents (primers, probes, and positive controls) and work protocols for these assays have been distributed by PAHO/WHO throughout the Region.

Additional molecular assays are available and can be performed on open (or “manual”) or closed platforms (*ie*, with kits that only work on automated, proprietary platforms). These include assays that have been approved for marketing by national regulatory authorities (in particular, those considered by WHO as SRA [Stringent Regulatory Authority] for its expedited pre-qualification of in-vitro diagnostic tests). WHO is also reviewing submissions for Emergency Use Listing (EUL) for assays for the detection of COVID-19 virus nucleic acid (17). Under the supervision of national health authorities and with the technical support of national public health laboratories and National Influenza Centers, these tests can be used in health care settings with the required capacity, or in decentralized laboratories.

Implementation and interpretation

Although the recommendation for laboratory confirmation of cases is to detect two different genetic targets (E followed by RdRP, as described above for the Charité protocol), once COVID-19 virus circulation is established and widespread in a given area/country, it is not longer necessary to run the PCR for both genes. Thus, confirmation through the detection of a single genetic target can be implemented, if the curves and other quality assurance parameters are optimal. Either E or RdRP genes can be used for the diagnosis; nevertheless, the E gene PCR has demonstrated slightly higher sensitivity, so we recommend prioritizing the E gene as the selected target.

Molecular detection of COVID-19 virus using well-designed protocols is usually very specific; thus, a positive result confirms the detection of the virus. On the contrary, a negative result might not always mean the absence of COVID-19 virus infection (9). Several reasons might explain a negative result in a person infected with COVID-19 virus, mainly:

- Poor sample quality, handling, transportation and/or storage (to control for this, the qualitative detection of a human housekeeping gene [eg, RNase P (18)] can be performed).
- Poor/failed sample extraction, presence of PCR inhibitors in the extracted RNA (to control for this, an extraction control can be used, or the detection of a housekeeping gene undertaken as mentioned above).
- The sample was collected at a time where the patient was not shedding sufficient amounts of virus, for instance very early or very late during infection (this point is particularly relevant as the dynamics of the viral presence in different sample types has not been fully established).
- As with any molecular detection assay, virus mutations in the regions that are targeted by the assays might affect the sensitivity of the detection.

Serological methods

Assays based on the detection of IgM / IgG antibodies can support outbreak investigation and seroprevalence studies. Several assays (both ELISA and rapid diagnostic tests) are available for the detection of IgM / IgG antibodies and are marketed for the detection of COVID-19 virus infections. However, to date, these tests are not recommended for use.

These tests may be limited due to cross-reactivity with other coronaviruses that are normally present in the community and that make the interpretation of results difficult (19). Furthermore, the dynamics of antibody response and production during the different stages of infection are not yet fully established at present, which further limits the use of these tests. Some studies have shown that during the first 6-7 days from the onset of symptoms, less than 40% of patients have detectable antibodies (20). Thus, serological tests should not be used to rule out a case during the first days of illness. Likewise, the detection of antibodies after day 7 only indicates previous contact with the virus but does not confirm the presence and shedding of the virus. The antibodies detected could result from a previous infection and not from the acute infection for which the diagnosis is being required.

Many commercial products are being marketed for the detection of antibodies (IgM and/or IgG) induced by COVID-19 virus infection, including rapid diagnostic tests (RDTs). Any such test should be validated and its performance in terms of specificity and sensitivity assessed. Currently and at the request of WHO, evaluation and eventual validation processes are underway for some of these tests. However, until now, none has an independent validation and therefore caution should be exercised in their use. Furthermore, the use of rapid tests is not recommended since (in addition to what was previously expressed), these types of tests might have low sensitivity (see below). For these reasons, antibody detection is not considered (as yet) an appropriate test for confirmation or diagnosis of COVID-19 cases. In house serological protocols are also under development in several laboratories.

Antigen detection

During the first days after symptom onset (approximately 1 to 5), viral proteins are generated that can be detected by different tests (*eg*, ELISA, immunofluorescence). In general, this type of assays has acceptable specificity (depending on the assay), therefore its detection can be used as a confirmation criterion (in conjunction with the case definition, the clinical history, and the epidemiological history) and to make public health decisions (*eg*, isolation).

However, the dynamics of production and secretion of these proteins (antigens) has not been established, therefore a negative result (at any stage of infection) should not be used as a criterion to rule out a case, and therefore other criteria must be taken into account.

Rapid diagnostic tests (RDTs)

So far there are no rapid diagnostic tests (immunochromatography or colloidal gold detection) that have been authorized by competent regulatory authorities and/or have been formally validated. In general, these types of tests have low sensitivity. Therefore, their positive predictive value is good (they can be used to rule in cases), but their negative predictive value is low (they should not be used to rule out cases). Also, the limitations described above for serological tests and antigenic detection apply to RDTs.

Testing Algorithm

Testing for COVID-19 virus should be considered for patients who fit the case definition (10). Laboratories should continue to use the influenza testing algorithm recommend by PAHO for routine influenza surveillance and unusual SARI cases.

Strengthening of laboratory capacity and networks

All national public health laboratories, including NICs, with molecular diagnostic capacity have implemented COVID-19 virus detection. Laboratories are urged to ensure the availability of human resources and generic supplies (eg, extraction kits and RT-PCR enzymes) for the detection of COVID-19 virus, and plan for a surge in laboratory testing.

Additionally, sequencing platforms may be used for COVID-19 virus genetic characterization in laboratories with Sanger or Next Generation sequencing capacity. These laboratories are encouraged to timely sequence positive samples and share genetic information through the Global Initiative on Sharing All Influenza Data Platform (GISAID). PAHO is working on establishing and strengthening a COVID-19 genomic sequencing network in the Americas region to make genomic data timely available.

Countries with no molecular diagnostic capacity to implement COVID-19 virus detection should send suspected clinical samples (strictly fitting the case definition) to a reference laboratory. The list of WHO reference laboratories providing confirmatory testing is available at:

<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>

In the Americas, to date there are two WHO reference laboratories for COVID-19 virus:

- Respiratory Viruses Diagnostic Laboratory, US CDC, Atlanta, USA.
- Respiratory Viruses Laboratory, Fiocruz, Rio de Janeiro, Brazil.

PAHO should be contacted before referring samples to WHO reference laboratories.

Countries with no molecular diagnostic capacity to implement COVID-19 virus detection but intending to establish such capacity can contact PAHO for guidance and support.

Data reporting

According to the International Health Regulations (IHR), all COVID-19 confirmed cases should be notified in 24 hours through official IHR channels (10).

Additionally, all positive and negative results for COVID-19 must be reported in the FluNet database that is sent weekly to PAHO/WHO. Updated FluNet spreadsheets with the addition of a new column for COVID-19 reporting have been sent to the countries to replace the previous version. Additional information might be obtained through flu@paho.org.

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