

Laboratory Guidelines for Detection and Diagnosis of the Novel Coronavirus (2019-nCoV) Infection

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Coronaviruses are a group of highly diverse RNA virus 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) (2) (3). There are endemic human coronavirus as the alphacoronavirus 229E and NL63 and betacoronaviruses OC43 and HKU1 that can cause influenza-like illness or pneumonia in humans (1) (3). However, two zoonotic coronavirus 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) (4) (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, but it is distinct from SARS-CoV and MERS-CoV (6) (7). The complete genome sequence of this new agent has been released and different detection protocols have been developed but not fully validated yet. However, in light of the possible introduction of a suspected case related to 2019-nCoV in the America region, the Pan American Health Organization / World Health Organization (PAHO/WHO) recommends to Member States to ensure their timely identification, the shipping of samples to National and reference laboratories and the implementation of the molecular detection protocol for 2019-nCoV, according to the laboratory capacity.

WHO have published on 17 January 2020 an updated of the interim guidance for Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases available at: <https://www.who.int/health-topics/coronavirus/laboratory-diagnostics-for-novel-coronavirus>.

Information on Suspected case definition; specimen collection and shipment; effective usage of global laboratory networking; testing of 2019-nCoV in reference laboratories; and reporting of cases and test results can be found in this interim guidance.

Sample collection and proper shipment

Samples should be collected by trained personnel and considering all biosafety instructions including the use of personal protective equipment appropriate for respiratory viruses.

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 transport medium). Although sampling of asymptomatic contacts on routine basis is not recommended, if it is considered necessary according to national guidelines, upper respiratory samples should be considered.

Samples should be kept refrigerated (4-8 °C) and sent to the laboratory (central, national or reference) 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). Shipment of suspicious samples to reference laboratories or collaborating centers outside the country and by air must ensure compliance with all international standards (IATA) for Biological Substances Category B.

Laboratory testing

WHO have made available some molecular diagnostic protocols on *the WHO Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases* webpage. Neither the names of vendors or manufacturers included in the protocols are preferred/endorsed by WHO. Also, these protocols have not yet been validated through WHO process.

Also, the US Centers for Disease Control (CDC) has developed a protocol for the nCoV detection. This protocol can be accessed on the following link:

<https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-for-detection-instructions.pdf>

Currently, PAHO is working to implement the first protocol made available by WHO, developed by the Charité Hospital, Berlin Germany. This protocol has been published and can be accessed on the following link:

<https://www.eurosurveillance.org/docserver/fulltext/eurosurveillance/25/3/eurosurv-25-3-5.pdf?expires=1580420806&id=id&accname=guest&checksum=D2E250BE3914A24D9381FC9538EFF11D>

This protocol is based on the detection of 3 different markers: genes N, E and RdRp. The assays for the genes E and N are intended as screening protocols to detect any bat-associated beta-coronavirus (do not detect common human coronavirus); the RdRp is specific for SARS and SARS-like coronavirus (including the 2019-nCoV).

Therefore, for a routine workflow, it is suggested to run first the N **or** the E gene assay first (it is not necessary to run both) as the screening tool, followed by confirmatory testing with the RdRp gene assay (8).

Testing Algorithm

Laboratories should continue to use the influenza testing algorithm recommend by PAHO for routine influenza surveillance and unusual SARI cases.

Testing for the nCoV should be considered **only for patients who fit the case definition, once influenza and avian influenza have been ruled out**. The interim case definitions for epidemiological surveillance and its updates are available at [https://www.who.int/internal-publications-detail/surveillance-case-definitions-for-human-infection-withnovel-coronavirus-\(ncov\)](https://www.who.int/internal-publications-detail/surveillance-case-definitions-for-human-infection-withnovel-coronavirus-(ncov))

Strengthening of Laboratory Capacity and Sample Flow

To strengthen the laboratory response capacity for nCoV in the Americas and considering the heterogeneity of National Influenza Centers and Influenza National Laboratories throughout the Region, PAHO has proposed 3 possible response scenarios:

1- Laboratories with no molecular diagnostic capacity

Laboratories with no molecular diagnostic capacity to implement the nCoV molecular protocol should send suspected clinical samples (strictly fitting the case definition) to a reference laboratory.

PAHO regional office should be consulted before referring nCoV suspected clinical samples to reference laboratory.

2- Laboratories with molecular diagnostic capacity

2a. Laboratories with molecular diagnostic capacity to implement nCoV molecular detection

Laboratories with the molecular diagnostic capacity might consider implementing one of the two nCoV molecular detection protocols as cited above.

Primer and probes sequences for oligonucleotides synthesis and bench protocols are available for each cited protocol*.

****In order to support the laboratories of the Americas Region, PAHO will provide positive controls (available soon) useful (so far) only for the protocol Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany.***

Positive samples by molecular detection should be immediately notified through official IHR channels. PAHO should be consulted for referring nCoV positive samples to a reference laboratory.

2b. Laboratories with molecular diagnostic and sequencing capacity

Laboratories with molecular diagnostic capacity might consider implementing one of the two nCoV molecular detection protocols made available by WHO as cited above.

Primer and probes sequences for oligonucleotides synthesis and bench protocols are available on each cited protocol. *

Additionally, sequencing protocols may be used for nCoV specific identification in laboratories with Sanger or Next Generation Sequencing capacity **only in PCR positive samples**.

Importantly, positive samples by molecular detection should be immediately notified through official IHR channels.

Laboratories with sequencing capacity are encouraged to timely sequence positive samples and share genetic information through the Global Initiative on Sharing All Influenza Data Platform (GISAID) and GenBank.

PAHO should be consulted for referring nCoV positive samples to a reference laboratory.

2c. Laboratories with molecular diagnostic not to implement nCoV molecular detection

Laboratories with molecular diagnostic capacity but with not capacity to synthesize the required oligonucleotides or implement molecular diagnostic for the nCoV, should follow the guidance for Laboratories with no molecular diagnostic capacity (item 1).

Shipping samples to the reference laboratory at CDC

It is important to keep in mind that the CDC reference laboratory for the nCoV is not the WHO Influenza Collaborator Center at CDC and samples should **not** be sent as routine influenza samples to CDC. The reference laboratory in the America region for nCoV suspected samples is the Respiratory Viruses Diagnostic Laboratory at US-CDC.

For logistic and shipping information of nCoV suspected samples to CDC, PAHO should be contacted.

The results obtained at the National Influenza Center or the National Public Health Laboratory should be immediately informed according to the established channels to ensure the necessary actions. As a reference, the laboratories have the **option** to send the first negative samples (up to 5 samples) and the first positive samples (up to 10 samples) to the CDC laboratory.

References

1. **Hui, DSC and Zumla, A.** Severe Acute Respiratory Syndrome - Historical, Epidemiologic; and Clinical Features. [book auth.] HW Boucher, A Zumla and DSC Hui. *Emerging and Re-emerging Infectious Diseases - Clinics Review Articles* . Philadelphia : Elsevier, 2019, pp. 869-889.
2. **Drosten , C, et al.** Severe acute respiratory syndrome: identification of the etiological agent. *Trends Mol Med.* 2003, Vol. 9, pp. 325-7.
3. **El, Azhar, et al.** The Middle East Respiratory Syndrome (MERS). [book auth.] Boucher HW, Zumla A and DSC Hui. *Emerging and Re-emerging Infectious Dseases - Clinics Rebiw Articles*. Philadelphia : Elsevier, 2019, pp. 891-905.
4. **de Wit, E, et al.** SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology.* 2016, Vol. 14, pp. 523-524.
5. **R, Hilgenfeld and M, Peiris.** From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. *Antiviral Res.* 2013, Vol. 100, pp. 286-95.
6. **Organization, World Health.** Laboratory testing of human suspected cases of novel coronavirus (nCoV) infection - Interim guidance. *WHO/2019-nCoV/laboratory/2020.1.* [Online] January 17, 2020. <https://www.who.int/health-topics/coronavirus/laboratory-diagnostics-for-novel-coronavirus>.
7. **GISAID.** Newly discovered betacoronavirus, Wuhan 2019-2020. *GISAID EpiFlu - Global Initiative on Sharing All Influenza Data.* [Online] January 2020. <https://platform.gisaid.org/epi3/frontend#414223>.
8. **Corman, VM, et al.** Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020, Vol. 25, p. 2000045.