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Editorial

Laboratory Testing COVID 19

In an ongoing pandemic it is critical to track the virus, understand pathogenesis and epidemiology, manage the cases and suppress transmission that requires effective laboratory testing. 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).

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. CoVs belong to the subfamily Coronavirinae in the family of Coronaviridae of the order Nidovirales, and this subfamily includes four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronaviru.[3]

The primary objective is to test all suspected cases in an effort to detect first cases in new areas or settings as rapidly as possible, and take immediate measures to prevent further spread in that region. For this it is important to improve and increase the number of laboratories capable of performing the recommended tests. Specimen handling for molecular testing would require BSL- 2 or equivalent facilities.

Specimen collection

- 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 however there will be high risk of aerosolization hence strict infection prevention and control measures should be taken.
- Autopsy material including lung tissue.
- 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.[4,5] The duration and frequency of shedding of COVID-19 virus in stool and potentially in urine is unknown.

Handling and transportation of specimen

Correct handling of specimens during transportation is important to minimize pre-analytic errors. Specimens which can be delivered promptly to the laboratory can be stored and transported at 2- 8°C. When there is likely to be a delay in specimens reaching the laboratory use of viral transport medium (VTM) containing antifungal and antibiotic supplements is recommended. Avoid repeated freezing and thawing of specimens. According to WHO guideline for Laboratories if VTM is not available sterile saline may be used in place of VTM and has to be frozen to - 20°C or ideally -70°C and transported on dry ice if further delays are expected. It is important to avoid repeated freezing and thawing of specimens.[6]

Laboratory testing

- **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. A number of factors could lead to a negative result in an infected individual, including poor quality of the specimen, inadequate material, specimen collected late or very early in the infection, specimen not handled and shipped appropriately and 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

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specimens, including from the lower respiratory tract if possible, should be collected and tested.

- **Rapid diagnostic tests based on host antibody detection:** This test detects the presence of antibodies in people believed to have been infected with COVID-19.[7] Antibodies are produced over days to weeks after infection with the virus. The strength of antibody response depends on several factors, including age, nutritional status, severity of disease, and certain medications or infections like HIV that suppress the immune system.[8]

  Studies suggest that the majority of patients develop antibody response only in the second week after onset of symptoms.[7] This means that a diagnosis of COVID-19 infection based on antibody response will often only be possible in the recovery phase, when many of the opportunities for clinical intervention or interruption of disease transmission have already passed. Antibody detection tests targeting COVID-19 may also cross-react with other pathogens, including other human coronaviruses [7] and give false-positive results. Lastly, there has been discussion about whether RDTs detecting antibodies could predict whether an individual was immune to reinfection with the COVID-19 virus. There is no evidence to date to support this. For clinical diagnosis, however, such tests have limited utility because they cannot quickly diagnose acute infection to inform actions needed to determine the course of treatment. Some clinicians have used these tests for antibody responses to make a presumptive diagnosis of recent COVID-19 disease in cases where molecular testing was negative but where there was a strong epidemiological link to COVID-19 infection and paired blood samples (acute and convalescent) showing rising antibody levels. Based on current data, WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance.

- **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 counter measures, including diagnostic tests. Virus whole genome sequencing can also inform molecular epidemiological 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.[6]

- **Viral culture:** Virus isolation is not recommended as a routine diagnostic procedure.[6]

Many aspects of the virus and disease are still not understood till date. Viral dynamics like optimal timing and type of specimen for laboratory testing, immunologic response, relationship between viral concentration and disease severity, duration of shedding, validation of serological assays as well as comparative study with molecular tests and mutations have to be understood. A better understanding will be needed to provide improved guidance.

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