JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH | Center for Health Security

Diagnostic Testing for 2019-nCoV

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Diagnostic testing for the novel coronavirus 2019-nCoV is undertaken using 2 approaches: whole genome sequencing and real-time reverse transcriptase PCR (rRT-PCR). Sequencing was used primarily in the early days of the outbreak for initial identification of this novel virus and is largely a tool of viral discovery. Currently, almost all diagnostic testing for nCoV is done using rRT-PCR.

A person in the United States can be tested for 2019-nCoV when he or she meets the US Centers for Disease Control and Prevention (CDC) criteria for a 2019-nCoV patient under investigation (PUI). These criteria include both clinical features and epidemiologic risk (ie, travel history or history of close contact with a confirmed case).[1]

Criteria as of January 30, 2020

Clinical Features	and	Epidemiologic Risk
*Fever **and** symptoms of lower respiratory illness (eg, cough, difficulty breathing)*	and	*In the last 14 days before symptom onset, a history of travel from Wuhan City, China.* – Or – *In the last 14 days before symptom onset, close contact with a person who is under investigation for 2019-nCoV while that person was ill.*
*Fever **or** symptoms of lower respiratory illness (eg, cough, difficulty breathing)*	and	*In the last 14 days, close contact with an ill laboratory-confirmed 2019-nCoV patient.*

*Table from US CDC: https://www.cdc.gov/coronavirus/2019-ncov/clinical-criteria.html.

Sample Collection and Handling Practices

When a patient is suspected of having 2019-nCoV infection based on the criteria above, and typically after other common respiratory viruses have been ruled out, healthcare providers in the United States are asked to report that patient as a PUI to infection control personnel at their healthcare facility and to their state health department. State health departments then report the PUI to the CDC Emergency Operations Center (EOC). CDC's EOC then assists in collection, storage, and shipping of specimens for diagnostic testing. CDC recommends collection of 3 specimen types: lower respiratory, upper respiratory, and serum specimens.[2]

CDC guidance states that clinical laboratories should not attempt viral isolation from 2019-nCoV PUI samples. According to CDC, "virus isolation in cell culture and initial characterization of viral agents recovered in cultures of 2019-nCoV specimens are NOT recommended at this time, except at a BSL3 facility."[2]

CDC further states that standard biosafety work practices should be used for pathologic examination, molecular analysis, electron microscopic studies, routine examination of cultures, routine staining and microscopic analysis of fixed smears, final packaging of specimens for transport, and inactivated specimens.[2] Class II biosafety cabinets should be used to perform: aliquoting and/or dilution of specimens, inoculating culture media, performing diagnostic tests (that do not involve propagation of viral agents), nucleic acid extraction procedures, and preparation and chemical- or heat-fixing of smears for microscopic analysis.[2]

Genetic Sequencing

Now that the virus has been identified, most sequencing is being undertaken to further research in order to characterize the virus and monitor for viral mutation, not for clinical diagnosis. However, some sequencing is also being done to generate real-time epidemiologic information. For example, sequencers from Oxford Nanopore are being used in China as part of the ARTIC project to develop "an end-to-end system for processing samples from viral outbreaks, to generate real-time epidemiological information that is interpretable and actionable by public health bodies." The ARTIC network has released a set of materials to assist in sequencing (using Oxford Nanopore equipment).[3] China CDC has also been sequencing samples via Oxford Nanopore products (specifically MinION) to investigate clusters in this outbreak.[4]

PCR Testing

Protocols for rRT-PCR testing developed by Germany, Hong Kong, China CDC, Thailand, and Japan have been posted to the WHO website,[5] and the protocol for testing in the US has been posted to CDC's site.[6] Cherité Universitätsmedizin Berlin developed the first assay and protocol.

US Protocol: As of January 27, 2020, all rRT-PCR tests on PUIs for 2019-nCoV infection in the United States are being run at the CDC in Atlanta. But in the coming weeks, CDC anticipates "sharing these tests with domestic and international partners through the agency's International Reagent Resource."[7]

- rRT-PCR primers and probes have been published by CDC: https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf
- Materials and reagents required to run the PCR tests are listed here: https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-for-detection-instructions.pdf

Commercial RT-PCR tests kits, described publicly as of January 23,[8] include kits from BGI (China), Co-Diagnostics (US), and Altona (Germany). BGI has already worked with the Chinese CDC and other Chinese authorities to issue test kits to hospitals and local health authorities there. The company is also working with Hong Kong and Taiwan, Brunei, Thailand, Nigeria, and South Africa to supply kits. Co-Diagnostics has completed initial design of an rRT-PCR test kit, and Altona has begun development of a test kit for 2019-nCoV detection. Neither of these kits has been put into use yet in the US or elsewhere.

Rapid Point-of-Care Tests

As of January 27, 2020, there have been no announcements of the development of non-PCR-based rapid diagnostic tests. Of companies that have rapid PCR-based assays:

- GenMark Dx has not announced anything related to 2019-nCoV.
- BioFire has not announced anything related to 2019-nCoV.
- Cepheid has not announced anything; however, their CMO for China recently co-authored an article on the virus in the *Journal of Medical Virology*.[9]
- Chembio has not announced anything related to 2019-nCoV.

Regulatory Considerations

The Food and Drug Administration launched a landing page to provide information to the public and to product developers on the agency's response to the nCoV outbreak.[10] The page includes an email address for diagnostic test sponsors interested in applying for an Emergency Use Authorization.

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