



PCR Diagnostic Testing for SARS-CoV-2

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Diagnostic testing for the novel coronavirus SARS-CoV-2 is undertaken using 4 approaches: whole genome sequencing, real-time reverse transcriptase PCR (rRT-PCR), antigen testing, and serology.

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, the majority of diagnostic testing for SARS-CoV-2 is done using rRT-PCR. Serologic tests that detect prior infection are now being developed and are discussed in another fact sheet. In rare circumstances, viral cultures can also be used, but they are not normal tools in clinical practice and are not covered here.

A person in the United States can be tested for SARS-CoV-2 by a physician's order when that person has symptoms consistent with COVID-19, the disease caused by the SARS-CoV-2 virus. Updates to guidance include prioritization of cases in areas where testing resources may be limited.¹ The CDC recommends that physicians use their best judgment in determining if a patient meets testing criteria and that fever, acute respiratory illness, and shortness of breath are typical symptoms. The current categories of testing are:

1. Symptomatic individuals: while the common symptoms of COVID-19 include fever, coughing, and sore throat, physicians should familiarize themselves with less common symptoms and typical symptoms in children.
2. Asymptomatic individuals with known or suspected exposure to SARS-CoV-2: These individuals should be tested to control outbreaks as quickly as possible. This may be broadened to all suspected contacts of an individual with SARS-CoV-2 infection if it is in a high risk environment where the virus could spread quickly.

Asymptomatic individuals with no known exposure to SARS-CoV-2: this testing is primarily in high risk environments. High risk environments can include professions that have close contact with individuals at high risk for severe COVID-19 (such as nursing homes), correctional facilities, homeless shelters, and workplaces with high population density performing essential services (such as meat processing plants). This testing of asymptomatic individuals should be coordinated with local health departments.

In areas with limited testing resources, the CDC has listed the following priorities for testing individuals:

1. Hospitalized patients, and close contacts of these patients (health care professionals)
2. Individuals at higher risk for severe disease
3. Individuals in communities experiencing high numbers of COVID-19 hospitalizations

At the time of publication, individuals who are asymptomatic and are not in high risk environments are not a priority for testing.

PCR Testing

Protocols for rRT-PCR testing developed by several countries and entities, including [Germany](#), [Hong Kong](#), [China CDC](#), [Thailand](#), and [Japan](#), have been posted to the WHO website,² and the protocol for testing in the [United States](#) has been posted to [CDC's site](#).³ Charité Universitätsmedizin Berlin [developed](#) the first assay and protocol.

US Protocol: rRT-PCR tests for SARS-CoV-2 infection in the United States are being run at the CDC in Atlanta for jurisdictions that do not have local testing available. The CDC has published a list of diagnostic rRT-PCR tests that have Emergency Use Authorization (EUA) approval.⁴

- rRT-PCR [primers and probes](#) have been published by CDC.
- Materials and reagents required to run the rRT-qPCR tests are listed [here](#).

Commercial, hospital-based, and academic rRT-PCR tests kits are now widely available.

Genetic Sequencing

Now that the virus has been identified, most sequencing is being undertaken 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).⁵ China CDC has also been sequencing samples via Oxford Nanopore products (specifically MinION) to investigate clusters in this outbreak.⁶ In the United Kingdom, the COVID-19 Genomics UK Consortium (COG-UK) was recently established to sequence the genomes of SARS-CoV-2.⁷ Worldwide efforts to sequence samples and establish phylogenies of the virus can be found on NextStrain, sponsored by GISAID.⁸ One EUA approved diagnostic test utilizes next generation sequencing (NGS) of samples to rapidly sequence and analyze patient samples.

Biosafety Practices

Characterization of viral agents recovered in cultures of SARS-CoV-2 specimens should be conducted only in a Biosafety Level 3 (BSL-3) laboratory using BSL-3 practices. 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.⁹ Class II biosafety cabinets should be used to perform sample collection that may create aerosols or droplets. If a Class II biosafety cabinet is not available, then precautions such as using PPE, splash shield, centrifuge safety cups, and sealed centrifuge rotors should be maintained.⁹

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 SARS-CoV-2 outbreak.¹⁰ The page includes an information for diagnostic test sponsors interested in applying for an Emergency Use Authorization.

References

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