

Amanda Kobokovich, MPH and Rachel West, PhD

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## Background

Diagnosis of COVID-19 involves detecting an active infection of SARS-CoV-2. Active infection can be assessed through detecting viral genetic material (RNA) or viral proteins (antigens).<sup>1</sup> Diagnostics include molecular tests, such as nucleic acid amplification tests, and rapid antigen tests. To date, over 200 molecular and antigen diagnostic tests have received Emergency Use Authorization (EUA) from the US Federal Food and Drug Administration (FDA).<sup>2,3</sup>

## Molecular diagnostics

Molecular diagnostic tests detect viral RNA, amplify specific genetic sequences that are unique to the virus, and produce a readable output that can be interpreted by laboratory experts, healthcare providers, or the patient.<sup>4</sup> The amplification step allows the test to identify even low levels of viral infection, such as those found at the beginning of an active infection. The most common type of COVID-19 diagnostic test is real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). These tests were created to be highly specific to the SARS-CoV-2 virus, thereby minimizing false positive results, and to detect low viral loads in respiratory samples.<sup>5</sup> These types of tests require trained research personnel to run samples and interpret results. Although rRT-PCR tests can take 2 to 4 hours to complete in a laboratory environment, return of results can take much longer due to processing times and high volumes of patient samples. In comparison, RT-LAMP (loop-mediated isothermal amplification) and CRISPR-based tests can take less than an hour to complete, but these test types are less commonly found on the FDA EUA lists.<sup>4</sup>

## Antigen diagnostics

Tests that detect viral antigens do not amplify these proteins, they only identify what is already present in a patient sample.<sup>6</sup> Antigen tests detect proteins unique to the virus that are shed in patient tissues, like the nostrils or saliva. Usually, rapid antigen tests have labeled antibodies that are specific to a certain viral protein, such as the nucleocapsid. These antibodies then may be measured using light output, or a color change, to detect a positive result.<sup>7</sup> Rapid antigen tests usually take only 15 to 30 minutes, but some require specialized equipment to read the results. For rapid antigen tests that are approved for point-of-care use, they must be specially tested to ensure their feasibility in that type of environment.<sup>8</sup>

## Other diagnostic tools

Certain diagnostics rely on genomic sequencing, sometimes referred to as next-generation sequencing (NGS).<sup>9</sup> NGS may be used in a diagnostic or research environment. NGS diagnostics not only amplify and detect SARS-CoV-2, they also sequence the virus genetic material present in the sample. These types of diagnostics are very specific and are particularly useful to identify viral strains or variants present in a patient sample. NGS can be leveraged to monitor for novel variants and inform other diagnostics or vaccines.<sup>10</sup> Although NGS diagnostics can take only a few hours to produce a result, they typically require specialized equipment, trained personnel, and software to analyze the data.



# Diagnostic Testing for COVID-19

In some cases, researchers may seek to isolate the virus from a patient sample and culture it in a lab environment. This method is useful to determine the viral titer (or amount of replicating virus) of a sample, which is often of interest for research use.<sup>11</sup> Culturing a virus takes highly trained personnel, adequate biosafety levels, and specialized reagents. In addition, virus culturing can help provide samples of new strains or variants of a virus for research use.

## Emergency Use Authorization

Most COVID-19 diagnostics are available under EUA, which is an accelerated, conditional authorization for use during a public health emergency to help detect and combat a new disease that poses a significant threat.<sup>12</sup> First established in 2007, the EUA process allows for manufacturers to market new devices on an accelerated timeline.

FDA EUAs are not FDA approvals. While an EUA can allow a manufacturer to market a test and an individual or healthcare provider to use a test, an FDA EUA is not equivalent to an FDA approval. An EUA, granted after review of a test, provides temporary, emergency use of a test. Each type of COVID-19 test must meet a specific set of requirements to qualify for an EUA. These thresholds are quality controls. While the test may not have full FDA approval, manufacturers must provide data that the test performs well and they must provide sufficient information on protocols, testing platforms, and other relevant details to the FDA.<sup>8</sup> Any test that does not have an EUA has not had the manufacturer provide this information to the FDA or had any independent review of the test.

Type of Test	Time to Results	What It Tells Us	Limitations
Rapid antigen test <sup>13</sup>	15 to 30 minutes	The presence of an active infection, by detecting specific viral proteins present in a patient sample. This is typically coupled with lateral flow assays to display results that can be read by eye.	Requires very careful design of synthetic antibodies, deep knowledge of viral proteins produced in various tissue environments, and may yield false negatives if the viral protein production is low.
rRT-qPCR <sup>14</sup>	2 to 4 hours	The presence of an active infection, by targeting specific gene sequences of SARS-CoV-2. This can be quantitative but is usually qualitative (yes/no). It typically has a very low limit of detection, around 100 viruses/mL.	Requires time to complete the test and trained personnel and special equipment to analyze the results.



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LAMP <sup>15</sup>	15 to 60 minutes	The presence of an active infection, by targeting specific gene sequences of SARS-CoV-2. This is typically qualitative (yes/no). It relies on specially designed primers that help create the looped structures needed for amplification. It is very rapid and does not always require special equipment (can be measured by eye in some cases). It has a very low limit of detection of 125 viruses/mL.	Designing the primers needed can be complex, and debris can interfere with the reaction. It is also difficult to quantify the results (level of viral infection).
Recombinase Polymerase Amplification <sup>16</sup>	15 to 60 minutes	The presence of an active infection, by targeting specific gene sequences of SARS-CoV-2. This is typically qualitative (yes/no). It relies on the recombinase enzyme. It is very rapid and does not always require special equipment. It has a very low limit of detection of 125 viruses/mL.	Designing the necessary primers can be complex, and debris can interfere with the reaction. It is also difficult to quantify the results (level of viral infection).
CRISPR-based diagnostics <sup>17</sup>	15 to 60 minutes	The presence of an active infection, by targeting specific gene sequences of SARS-CoV-2. This is typically coupled with LAMP, but this is not always necessary. Results are typically visible to the eye, not requiring special equipment.	Requires expert, specific design of components (ie, enzymes, primers, reporters).

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