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COVID-19 Testing: An Overview for Pharmacists

by Parmida Parvaz, PharmD

COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains an ongoing global pandemic, with millions of confirmed cases worldwide. Detection through diagnostic testing is vital in treating and controlling the spread of the virus. Since the identification of the viral genome, several testing methodologies have been developed. Testing can be classified into two categories: molecular testing and serology testing. Molecular tests detect the presence of virus and can signify an active infection, whereas serology testing detects viral antibodies, indicating a past infection and/or exposure.[1] While the timing of viral and antibody presence can vary between patients, a general depiction of the different phases of COVID-19 can be seen in Figure 1. With the urgent need for broader testing capacities, the Food and Drug Administration (FDA) has issued emergency use authorizations (EUAs) for a variety of in-vitro diagnostic devices to be used for the detection and management of COVID-19.[2] Furthermore, routine labs for patients hospitalized with COVID-19 can be utilized to direct therapy for those with more severe disease.

Molecular Testing

Real-time Reverse Transcriptase Polymerase Chain Reactions (rRT-PCR)

The Infectious Disease Society of America (IDSA) guidelines for the diagnosis of COVID-19 recommend nucleic acid amplification testing (NAAT) in patients with clinical suspicion of COVID-19. The rRT-PCR detects nucleic acids of SARS-CoV-2 RNA from upper and lower respiratory specimens.[4] The RNA of SARS-CoV-2 can be detected one to three days before symptoms onset, peaks within the first week of infection, and declines over time. However, detection of RNA does not always indicate infectiousness, as patients can have a prolonged duration of viral shedding. Viral RNA has been detected in respiratory specimens as long as 12 weeks after symptoms onset; however, replication-competent virus was not found in patients after 3 weeks of symptom onset.[1] The IDSA guidelines recommend collecting nasopharyngeal, mid-turbinate, or nasal swabs rather than oropharyngeal swabs or saliva specimens. This suggestion is based on several studies that have shown a lower viral load in the throat compared to the nose and thus a lower sensitivity of oral swabs.[4] While specificity is near 100% for different specimen types, sensitivity was found in oral to be 56%, nasal 95%, nasopharyngeal 97%, and mid-turbinate 100%.[5] There are also several home kits granted FDA EUAs that involve patient-collected samples of nasal or mid-turbinate swabs. While data are limited, similar rates of detection have been shown between healthcare provider-collected and patient-collected nasal or mid-turbinate swabs.[6] A study by Tu et al. compared nasopharyngeal samples collected by a healthcare worker to tongue, nasal, and mid-turbinate samples collected by patients, and found estimated sensitivities to be 89.8%, 94.0%, and 96.2%, respectively.[6] Asymptomatic patients have a greater chance of false negative test results. Data are limited and diagnostic test performance has not yet been established in asymptomatic patients.[7] It is assumed that the overall test sensitivity is between 75% and 95%. The average incubation time is estimated to be 5 days; therefore, 5 to 7 days after exposure would

FIGURE 1. Viral and Antibody Presence in COVID-19[3-4]

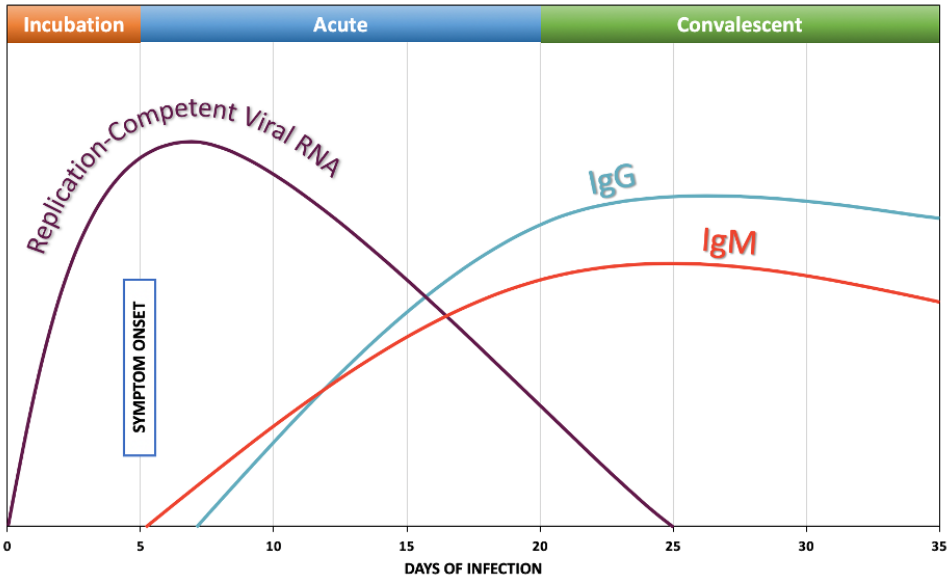

TABLE 1. COVID-19 Test Summary Table[2,4,8,9,10,21]

Test Type	Sensitivity	Specificity	Optimal Testing Time post-exposure	Advantages	Disadvantages
rRT-PCR	~98%	~95%	5-7 days	Gold standard	Inadequate sampling can skew results. Turnaround time may vary due to lab burden
LAMP	~95%	~99%	5-7 days	Rapid POC results, portable device	Complex primer design system, lack of data
Antigen	85-97%	100%	4-5 weeks	Less expensive, rapid results	Negative results should be confirmed with molecular test
Antibody	90-100%	95-100%	4-5 weeks	Determine population prevalence	Variable results based on patient and timing of test. Current evidence lacking in terms of correlation to actual immunity.

rRT-PCR = Real-time Reverse Transcriptase Polymerase Chain Reactions; LAMP = Loop-mediated Isothermal Amplification; POC = point of care

be a reasonable time to consider testing asymptomatic patients.[4] Currently, rRT-PCR remains the mainstay of diagnostic testing for COVID-19.

Loop-mediated Isothermal Amplification (LAMP)

As rapid point of care tests, an advantage of LAMP testing is that it can be conducted in a community care setting. The assay can produce results in 25 minutes from time of swab. A study by Österdahl and colleagues compared LAMP to repeated rRT-PCR results and found that the sensitivity and specificity was 80% for LAMP and 73% for rRT-PCR.[8] One example of isothermal amplification is Abbott's ID NOW™, which was granted FDA EUA and can produce results in 5 to 13 minutes. The device performance has been shown to be least 91.3% sensitive and 98.6% specific. Yet evaluations of this test have been highly controversial, with some researchers finding inaccuracies and high false negative rates.[9] Other rapid point of care tests, such as AQ-TOP COVID-19 Rapid Detection Kit ™ (SEASUN BIOMATERIALS Inc.), use reverse transcription-loop mediated isothermal amplification (RT-LAMP) technology. In a study by Huang and colleagues, 16 patient specimens that had been tested with rRT-PCR (8 positive, 8 negative) were also tested using RT-LAMP technology and showed identical results.[10] Studies thus far have been small in scale and unreliable, making LAMP testing less favorable than the rRT-PCR diagnostic tests.

Antigen Testing

Antigen tests are rapid, are typically cheap, and can result within minutes. The test

involves the binding of antibodies to targeted viral proteins and the generation of a signal that indicates detection of the antigen.[11] Two antigen tests for SARS-CoV-2 have received FDA EUA thus far. The downside of antigen testing is the weak sensitivity and specificity compared to nucleic acid testing. Quidel TM, a company that retains an EUA for its antigen test, reports that it meets the FDA's minimum 80% sensitivity requirement and is preparing a test that will reach 90% in the coming months.[12] On the FDA site for COVID-19 testing, there is a disclaimer stating that antigen tests cannot definitively rule out COVID and that a molecular test may be needed to confirm negative results.[2] Thus, while antigen tests can be more convenient and inexpensive, the results are less reliable than other methods of viral detection.

Serology/Antibody Testing

Serology tests detect the presence of an immune response and antibody production against the SARS-CoV-2. Antibodies are formed against viral antigens with IgM and IgG emerging concurrently, 5-7 days after symptoms onset.[3] In a study by Long and colleagues of 285 patients with COVID-19, 100% tested positive for antiviral IgG within 19 days of symptom onset.[13] Tests that detect IgM and/or IgG can determine whether an individual has been previously infected with COVID-19 and are not intended to replace a diagnostic test that indicates active infection. Results can be used to estimate the prevalence of viral exposure and infectivity within a defined population. The IDSA guidelines recommend testing for IgG 3 to 4 weeks after symptoms onset for highest sensitivity of results. Due to consistently higher sensitivity with IgG compared to IgM testing, antibody tests that detect IgG are recommended if there is need to assess for evidence of past COVID-19 infection.[4] Historically, serology tests have aided in identifying potential donors of convalescent plasma, which has been used as therapy for patients with severe COVID-19.[14] Presence of antibodies does not definitively indicate immunity, as there is much still not understood about protective immunity after COVID-19. In the same study by Long and colleagues, the authors compared immune response between asymptomatic and symptomatic patients and found that the symptomatic group had significantly greater IgG levels than the asymptomatic group in the acute phase (viral RNA present in respiratory specimen). It was also found that 40% of asymptomatic and 12.9% of symptomatic patients became seronegative for IgG in the early convalescent phase. The convalescent phase is the period at which clinical signs of illness have resolved, defined in this study as 8 weeks after discharge from hospital.[15]

A variety of serology test types

are available for commercial use. Neutralization tests measure the ability of antibodies to inhibit viral growth in the lab. Chemiluminescent immunoassay (CLIA) tests produce a fluorescent signal as viral proteins bind to antibodies. Enzyme-linked immunosorbent assays (ELISAs) use detector antibodies to signal viral antigen and patient antibody interactions. Lateral flow assays (LFAs) use patient samples over a membrane that contains the target antigen and leads to a colored display.[13] The FDA has given EUAs for several serology tests for IgG and/or IgM detection. The sensitivities range from 90% to100% with specificities of 95% to 100%.[16]

Laboratory Testing

Once a patient is diagnosed with COVID-19, therapeutic management can be determined by a variety of patient factors and test results. Severe systemic inflammatory response and respiratory failure are associated with increased mortality.[17] Biomarkers of inflammation can be used to direct treatment in select patient populations. Increased levels of C-reactive protein (CRP), interleukin 6 (IL-6), and erythrocyte sedimentation rate (ESR) have been associated with increased severity of disease.[18] The ElecsysTM (Roche Inc.) IL-6 immunoassay was granted FDA EUA and is used to measure interleukin-6 in human serum and plasma.[16] Such lab values can direct therapy toward immunomodulators such as tocilizumab, which acts as an IL-6 inhibitor.[19] Further laboratory tests can be utilized among hospitalized patients to detect COVID-19-associated coagulopathy, such as D-dimers, prothrombin time, platelet count, thromboelastography, and fibrinogen. These results can direct decisions for anticoagulation therapy for patients with severe COVID-19.[20] Additionally, liver and renal function tests are vital when remdesivir is being considered as therapy. Such tests are trended and interpreted along with clinical contents to help guide therapeutic decisions. While the body of data is constantly evolving for management of COVID-19, routine lab tests remain an important tool for creating a clinical picture and dictating appropriate treatment options.

Opportunities for Pharmacists

Pharmacists have played a pivotal role in patient care during the COVID-19 pandemic. Involvement in drug shortage management, development of treatment protocols, and interpretation of laboratory results are just some of the responsibilities pharmacists have taken on in the healthcare setting. Community pharmacists remain highly accessible health workers and have contributed in the education and screening of patients. The U.S Department of Health and Human Services has authorized pharmacists to order and administer COVID-19 tests. Furthermore, pharmacists will be vital in the immunization process when a COVID-19 vaccine becomes available. Pharmacists are frontline health workers with specialized knowledge of drugs and can provide patient care by utilizing these diagnostic and supporting tests for therapeutic management in their clinical practice.

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References

1. Centers for Disease Control and Prevention. Overview of testing for SARS-CoV-2. https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html Updated July 17, 2020. Accessed August 8, 2020.
2. U. S. Food and Drug Administration. Coronavirus testing basics. https://www.fda.gov/consumers/consumer-updates/coronavirus-testing-basics. Updated July 16, 2020. Accessed August 8, 2020.
3. Marca AL, Capuzzo M, Paglia T, et al. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. *RBMO*. 2020:00(0):1-17.
4. Infectious Disease Society of America. Guidelines on the diagnosis of COVID-19. https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/. Published May 6, 2020. Accessed August 8, 2020.
5. Wang X, Tan L, Wang X, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. *Int J Infect Dis*. 2020;94:107-109.
6. Tu YP, Jennings R, Cangelosi GA, et al. Swabs collected by patients or health care workers for SARS-CoV-2 testing. *N Engl J Med*. 2020;383(5):494-496.
7. Woloshin S, Patel N, Kesselheim AS. False negative tests for SARS-CoV-2 infection - challenges and implications. *N Engl J Med.* 2020;383(6):e38.
8. Österdahl MF, Lee KA, Lochlainn MN, et al. Detecting SARS-CoV-2 at point of care: preliminary data comparing Loop-mediated isothermal amplification (LAMP) to PCR. [published online April 4, 2020] medRxiv. doi.org/10.1101/2020.04.01.20047357
9. Basu A, Zinger T, Inglima K, et al. Performance of the rapid Nucleic Acid Amplification by Abbott ID NOW COVID-19 in nasopharyngeal swabs transported in viral media and dry nasal swabs, in a New York City academic institution. *J Clin Microbiol.* 2020;58(8):e01136-20.
10. Huang WE, Lim B, Hsu C, et al. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microb Biotechnol.* 2020;13(4):950–961.
11. Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J Clin Virol.* 2020;129:104455.
12. Service, RF. Coronavirus antigen tests: quick and cheap, but too often wrong? *Science*. 2020. doi:10.1126/science.abc9586.
13. Long QX, Lui BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med.* 2020;26(6):845-848.
14. U. S. Food and Drug Administration. EUA authorized serology test performance. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance. Updated August 01, 2020. Accessed August 8, 2020.
15. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med.* 2020;26(8):1200-1204.
16. U. S. Food and Drug Administration. In Vitro diagnostics EUAs. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-serological. Updated August 08, 2020. Accessed August 8, 2020.
17. Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *Int J Infect Dis.* 2020;96:467-474.
18. Centers for Disease Control and Prevention. Interim clinical guidance for management of patients with confirmed coronavirus disease (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html. Updated June 30, 2020. Accessed August 8, 2020.
19. Calabrese C, Rajendram P, Sacha G, Calabrese L. Practical aspects of targeting IL-6 in COVID-19 disease. [Published online October 7, 2020] *Cleve Clin J Med*. doi: 10.3949/ccjm.87a.ccc018
20. National institute of Health. Antithrombotic therapy in patients with COVID-19. https://www.covid19treatmentguidelines.nih.gov/adjunctive-therapy/antithrombotic-therapy/. Updated May 12, 2020. Accessed August 8, 2020.
21. Xpert® Xpress-SARS-CoV-2.[package insert]. Sunnydale, CA: Cepheid; 2020.