Considerations for Appropriate Use of SARS-CoV-2 Nucleic Acid Amplification Tests (NAAT), Point-of-Care (POC) Antigen Tests and Serologic Testing

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Summary

- The COVID-19 pandemic is still an ongoing public health threat. Correct identification and isolation of infected SARS-CoV-2 cases (symptomatic, pre-symptomatic and asymptomatic) is imperative to limit the spread of infections.[1-4]
- Effective programs to manage risk of COVID-19 spread in a population should include use of symptom checkers, social distancing, contract tracing, proper face masks, and access to testing when clinically appropriate. In this context, the use of less sensitive, but high-quality rapid SARS-CoV-2 testing, if used frequently (such as once per day or every three days), can play a role in detecting asymptomatic cases and preventing the spread of the virus in the workplace or community.
- If available, molecular SARS-CoV-2 NAAT assays, which include the commonly used polymerase chain reaction (PCR) test, should be performed in symptomatic individuals in most cases, and individuals should isolate themselves to prevent further spread of the disease pending receipt of the NAAT assay results. The CDC is also recommending asymptomatic individuals who have come into contact with someone with SARS-CoV-2 be tested 3-5 days after exposure.
- A positive antigen result in a symptomatic individual does not generally require confirmation with NAAT to make the diagnosis of SARS-CoV-2 infection. Antigen tests therefore may have a role when NAAT tests are not readily available.
- In accordance with CDC, serological testing does not replace virologic testing and should not be used to establish the presence or absence of acute SARS-CoV-2 infection.
- Existing antibody tests, including those that predict neutralizing antibody titers, may one day play a role in determining if natural infection or vaccine administration leads to a robust immune response and determination of the durability of that response. High-quality clinical studies can inform who should be prioritized for vaccine administration, or for vaccine boosters, should such an approach be needed based on emerging clinical data.
- As per the CDC, routine use of antibody testing is not currently recommended to assess for immunity to COVID-19 following vaccination or to assess the need for vaccination in an unvaccinated person. However, further epidemiological and clinical studies using neutralizing antibodies may help guide future therapies and booster vaccinations. Additionally, antibody testing may help determine whether individuals who are vulnerable or at higher risk of severe clinical outcomes from COVID-19 have already been infected and therefore have some level of protection against serious reinfection and transmission to others.

Background on COVID-19 Pandemic and Selection of an Appropriate Assay

The COVID-19 pandemic is an ongoing global public health crisis. In the United States, clinical laboratories have contributed tremendously to this pandemic response by deploying many technologies and 24-hour workflow to meet the evolving needs of COVID-19 patients.

SARS-CoV-2 infections can be transmitted from asymptomatic as well as pre-symptomatic and symptomatic individuals.[1-4]

As of July 2021, the Centers for Disease Control and Prevention (CDC) recommends that individuals who have symptoms of SARS-CoV-2, those who have had a known exposure to someone suspected or confirmed with SARS-CoV-2 and those individuals not fully vaccinated with an approved or authorized vaccine should be tested for SARS-CoV-2. Fully vaccinated individuals who have come into close contact with someone with COVID-19 should be tested 3-5 days following the date of their exposure. The CDC recommendations note that "although the risk that fully vaccinated people could become infected with COVID-19 is low, any fully vaccinated person who experiences symptoms consistent with COVID-19 should isolate themselves from others, be clinically evaluated for COVID-19, and tested for SARS-CoV-2 if indicated."

To limit community spread from infected asymptomatic and pre-symptomatic individuals, surveillance or screening testing programs at the appropriate frequency using accurate tests that provide quick results can help contain the spread of the virus. Symptom screening, testing, and contact tracing are strategies to identify people infected with SARS-CoV-2, so that actions can be taken to slow and stop the spread of the virus.

Assays to confirm the presence of the virus include those that detect SARS-CoV-2 nucleic acid and those that detect viral antigens. Molecular SARS-CoV-2 NAAT assays detect genetic material from the virus where many copies of the genetic material are generated in vitro using different types of amplification processes. NAAT assays include: polymerase chain reaction (PCR), transcription mediated amplification (TMA) and Loop-mediated Isothermal Amplification (LAMP). Antigen tests are immunoassays that detect the presence of a specific viral antigen(s). SARS-CoV-2 antigen assays are available as point-of-care (POC) assays as well as high throughput antigen assays that are intended to be performed on automated analyzers in laboratories accredited by the College of American Pathology or other approved accreditation organizations or otherwise certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). As amplification of the viral target is not a component of the antigen assays, these assays have lower analytic sensitivity (higher limit of detection) relative to NAAT assays.

Commercial molecular assays have been in place since March 2020 for assistance in diagnosing active SARS-CoV-2 cases. A positive result in a symptomatic individual, using an assay with higher analytic sensitivity (lower limit of viral material detection), such as one of the tests categorized as NAAT, assists in providing a definitive clinical diagnosis. Among individuals infected with SARS-CoV-2, approximately 33% to 45% are asymptomatic; [3,4] therefore, we propose to continue using any of the FDA Emergency Use Authorization NAAT tests as tests of choice in the symptomatic population, especially in those requiring hospitalization. Further, we recommend that the relevant federal agencies (e.g., CDC and FDA) provide more education regarding the appropriateness of using any of the NAAT tests for these needs, as there are many businesses, airlines and even countries that will only accept PCR methodology test results as valid based upon a belief that only PCR test results can be used to diagnose COVID-19. This misconception places an unnecessary burden on labs and resources that can be alleviated through enhanced federal communication.

Many SARS-CoV-2 antigen tests are now readily available and are clinically useful, if employed on a frequent recurring basis in congregate settings. Some of these applicable congregate settings would include workplaces, and colleges and other schools. New data demonstrate that the more frequently one is evaluated via antigen testing, the overall reliability of the testing increases (26). In certain situations, those asymptomatic individuals who do test positive using the antigen tests should have confirmatory testing using a NAAT methodology based on the CDC algorithm (Fig 1). Symptomatic individuals should isolate such that a diagnosis delay does not lead to further spread of the disease pending receipt of the confirmatory NAAT assay results. (Fig 2)

Antigen negative, asymptomatic individuals are unlikely to be infectious, but a negative antigen test does not exclude someone as being infectious at the time of antigen testing. Patients with a high pre-test probability of infection should have NAAT assay performed if antigen negative; however, for screening of asymptomatic patients, it is probably not necessary.The lower analytical sensitivity of antigen testing may be offset by more regular screening. [6,7,8] As POC assays can provide results within 15-30 minutes of testing an individual, use of such tests can help to provide rapid guidance for isolation in an asymptomatic or pre-symptomatic individual who tests positive. Care must be taken to follow the manufacturer instructions for use as false negative results have been reported when a POC antigen test with a high limit of detection is used.

The use of POC SARS-CoV-2 antigen assays as part of a surveillance or screening program can be an important addition to current safety protocols that can help to prevent virus spread and contain outbreaks.[9] POC assays backed up by appropriate NAAT assays can provide timely, actionable results to help supplement current safety measures, such as social distancing, wearing masks, temperature checks, symptom questionnaires, and handwashing, and may serve as a critical enabler of return to daily activities.

Frequency of Use and Efficacy for Antigen Tests

Transmission of SARS-CoV-2 appears to occur 2 to 14 days after exposure when the viral load peaks, and at this time individuals may remain asymptomatic. Asymptomatic transmission increases the need for more frequent testing rather than one-time testing to “rule out” an infection. Two modelling studies have been published related to the use of less sensitive antigen testing to detect SARS-CoV-2 with a recommended increased frequency of antigen testing in screening or surveillance programs.

Larremore, et. al. investigated the effects of surveillance testing strategies using POC antigen tests vs. NAAT/PCR tests at the population level.[10] They observed that testing frequency was the primary driver of population-level epidemic control, with only a small margin of improvement provided by using a more analytically sensitive test. Direct examination of simulations showed that with no surveillance or biweekly testing, infections were uncontrolled, whereas surveillance testing every day or every three days with either testeffectively attenuated surges of infections.

Paltiel, et. al. conducted an analytic modeling study to assess SARS-CoV-2 screening strategies to permit the safe reopening of college campuses in the United States.[11] They concluded that screening every two days using a rapid POC antigen test with low sensitivity, coupled with strict behavioral interventions such as wearing masks, maintaining social distancing and washing hands to keep Reproductive numbers (R) less than 2.5, is estimated to maintain a controllable number of COVID-19 infections and permit the safe return of students to campus. Their findings suggest testing frequency based on the various Reproductive numbers (R) scenarios as below:

- Base case: R of 2.5, screening every 2 days

- Worst case: R of 3.5, daily screening
- Best case: R of 1.5, weekly screening

Smith et. al. in a longitudinal study of 43 adults newly infected with SARS-CoV-2 used saliva and nasal swabs for quantitative reverse transcription polymerase chain reaction (RT-qPCR), Quidel SARS Sofia antigen fluorescent immunoassay (FIA), and live virus culture. They found that RT-qPCR tests are more effective than antigen tests at identifying infected individuals prior to or early during the infectious period. All tests showed >98% sensitivity for identifying infected individuals if they were repeatedly used at least every 3 days. Daily screening using antigen tests can achieve approximately 90% sensitivity for identifying infected individuals while they are viral culture positive.

Based on the findings of these studies, an effective surveillance or screening program can be considered as a dynamic program, testing more frequently when the R is higher and reducing the testing frequency when the situation is relatively under control. The CDC provides guidance on the frequency of testing based on the community indicators.[27]

Finally, it is sometimes difficult to employ such a program using rapid POC antigen tests, particularly if the group requiring testing is large. For example, POC testing may be disruptive in elementary school settings with children less than 12 years who are not vaccine eligible. Pod-pooled testing for infection in small groups may uncover any current infections. A collection of up to twenty-five nasal swabs, is tested at the group level instead of the individual level. It provides a cost-effective surveillance testing for K-12 schools. Individuals whose swab is part of a positive surveillance pod require follow-up diagnostic testing. The CDC recommends screening for students who are not fully vaccinated at least once per week if the community transmission is moderate to high and teachers and staff who are not fully vaccinated at least once per week irrespective of the community transmission. High-risk extracurricular activities such as activities that involve singing, shouting, band, or exercise, especially when conducted indoors require screening at least once a week if the community transmission is low and twice weekly for moderate to high transmission. Levels of community transmission are defined as total new cases per 100,000 persons in the past 7 days (low, 0-9; moderate 10-49; substantial, 50-99, high, ≥100) and percentage of positive tests in the past 7 days (low, <5%; moderate, 5-7.9%; substantial, 8-9.9%; high, ≥10%.)[28]

Role of Home-Based POC Testing

The U.S. Food and Drug Administration (FDA) had granted an Emergency Use Authorization (EUA) for home collection devices where after collection the sample is sent to the lab fortesting. There is one molecular prescription at-home test, three antigen prescription at-home tests, seven antigen over-the-counter (OTC) at-home tests and two molecular OTC at-home tests.[12]

Potential Role of COVID-19 Serologic Testing in Natural SARS-CoV-2 Infection and Vaccination

The FDA has not authorized the use of antibody tests to diagnose SARS-CoV-2 infection; however, serologic assays may be used to support[13] clinical assessment of persons who present late in their illnesses (>9–14 days after illness onset) when used in conjunction with viral detection tests, as well as in persons suspected to have a post-infectious syndrome caused by SARS-CoV-2 infection (e.g., Multisystem Inflammatory Syndrome in Children; MIS-C). In addition, surveillance antibody testing using a certain type of SARS-CoV-2 antibody test can reveal a more complete picture of SARS-COV-2's spread and its changes over time. This testing can provide a historical view, identifying individuals who may have been infected by SARS-CoV-2, including those who were asymptomatic. In addition, these tools can determine prevalence of infection in a population, particularly in underserved populations and

populations with limited access to testing.

Determining previous infection

Serologic assays for SARS-CoV-2 infection are also an important tool for surveillance and epidemiologic[14] studies,[1] as in understanding the transmission dynamic of the virus in the general population. Unlike direct viral detection methods, such as nucleic acid amplification or antigen tests that can detect acutely infected persons, antibody tests help determine whether the individual being tested was previously infected—even if that person never showed symptoms. Human studies have demonstrated that an individual may begin to develop a protective/neutralizing antibody response within a week after a NAAT/PCR confirmation of SARS-CoV-2 infection.[14] Additionally, there is compelling evidence that using total antibody or combined IgG/IgM assays offers the highest sensitivity of antibody detection.[15] Yangchun, in a study on optimizing diagnosis of COVID-19 from suspect cases by likelihood ratio of SARS-CoV-2 IgM and IgG found for suspected cases, after single detection of IgG and IgM antibodies, the clinician's confidence in the diagnosis of suspected patients as COVID-19 confirmed cases is between 86.26% and90.18%. After an IgM/IgG tandem test, the clinician's confidence in the diagnosis of suspected patients as COVID-19 confirmed cases increased to 99.15%.[16]

Assessing immune response, both before and after vaccination

The immunological basis for vaccination depends upon two central properties of the adaptive immune system: antigen specificity and memory. The antigenic targets used for the vaccines are spike proteins; therefore, they lead to antibodies against the spike protein only. Natural infection with SARS-CoV-2 tends to produce antibodies that can bind not just the spike, but also to other viral proteins such as nucleocapsid. Antibody tests targeting the nucleocapsid proteins will not be useful to measure the vaccine induced antibody response. Assessing the effectiveness of a vaccine is directly related to its ability to induce immunological response. Tests measuring anti-SARS-CoV-2 IgG concentrations and neutralizing antibody titers to the SARS-CoV-2 virus targeted to spike protein and receptor binding domains have been used in the phase 2 and phase 3 clinical trials to correlate with the efficacy of vaccines under development. There may or may not be a role for similar SARS-CoV-2 serological assays to determine the efficacy, durability, and the need for a booster dose.

Measurement of an immune response to a vaccine is usually determined by measuring the appearance and/or concentration of specific antibodies in serum. Semi-quantitative antibody assays (IgG and Total) and/or neutralizing antibody testing targeted to the correct antigenic domains of the SARS-CoV-2 virus can identify successful immune response, although the titer required for immune protection from infection/reinfection is not known at this time. With the availability of WHO International Standard (IS) for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136) accurate calibration of assays to a standard reference unit is now possible, thereby reducing inter-laboratory variation, and creating a common language for reporting data. It has an assigned potency for neutralizing Ab activity IU/ml and an arbitrary unit for binding antibody units (BAU)/ml, that IVD vendors can use to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity (e.g., anti-RBD IgG, anti-N IgM, etc.).[29] A positive antibody test result means that a person has antibodies from a previous SARS-CoV-2 infectionor a successful COVID-19 vaccination event(s).

Evaluation of persisting antibodies has been used to correlate with the duration of vaccine-induced immunity in other viral infections. Qualitative detection of IgG antibodies correlates with clinical protection in measles,mumps, rubella, hepatitis B, or varicella. For other viruses, quantitative detection of IgG is used to determine protection (e.g., hepatitis B). To determine a correlate of effectiveness and protection post-vaccination, a quantitative serologic IgG or total antibody assay, or a surrogate neutralization antibody assay, may potentially be used.

Importantly, post-vaccination testing is not routinely recommended at this time because the specific correlation of protective performance of the serological testing has not been established in COVID-19 vaccinees. A weak antibody response to 2-Dose SARS-CoV-2 mRNA Vaccine series in solid organ transplant recipients has been reported.[30,31] The FDA and CDC now recommend that moderately to severely immunocompromised people receive an additional vaccine dose intended to improve response to their initial vaccine series.[32] The use of post- vaccination antibody response as determined by serology testing, may help clinicians evaluate certain patients who may have a prior immune compromised condition, to determine whether their patient responded appropriately to a vaccination.

Duration and correlates of protective immunity to SARS-CoV-2

Dan et al. reported the duration of protective antibody to SARS-CoV-2 suggesting alevel of protection that may last up to 8 months when looking at all components of the immune system including IgG antibodies, B and T cell responses.[18]

More studies are needed to make a conclusive decision on the duration of protective immunity in SARS-CoV-2, but early results are encouraging.

Fewer COVID-19 reinfections have been demonstrated in people with positive SARS-CoV-2 antibodies.[19-21] A study published in *JAMA Internal Medicine* found among 3.2 million unique patients with an index antibody test, among more than 19,000 individuals after 90 days post antibody testing, only 0.3% of antibody positives had a positive NAAT compared with 3.0% of antibody negatives.[19] In the United Kingdom, among more than 12,000 healthcare workers, after 6 months only 2 asymptomatic PCR positive cases occurred in the antibody positive group vs 233 in the antibody negative group[20] and in a similar study from France, among more than 8,000 healthcare workers after 5 months only 3 symptomatic PCR positive cases occurred in the antibody positive group vs > 1,000 in the antibody negative group.[21]

It is important to note that antibody levels only represent one arm of the immune system and may serve as a surrogate for other immune response mechanisms, including T-cell responses and other aspects of the immune system. Therefore, waning antibody levels over time does not necessarily indicate that an individual lacks immune protection from the virus. If a person who was previously infected is later exposed to the virus again in the future, the robust B cell and T cell memory responses induced by primary infection suggest that reinfection severity, and potentially transmission, may be mitigated over the longer term.[36]

SARS-CoV-2 seroprevalence in a repeated cross-sectional study including more than 1.4 million blood donation specimens from a catchment area representing 74% of the US population, increased from 3.5% in July 2020 to 20.2% for infection-induced antibodies and 83.3% for combined infection- and vaccine-induced antibodies in May 2021. Seroprevalence differed by age, race and ethnicity, and geographic region of residence, but these differences changed over the course of the study. These data can supplement public health surveillance to identify groups potentially at higher risk of infection to implement public health prevention measures. Additionally, these approaches can inform infectious disease modeling and other research (e.g, estimating infection-mortality ratios, ecologic analyses of associations between population immunity and case incidence).[37]

SARS-CoV-2 Variants

Viruses constantly change through mutation, and new variants of a virus are expected to occur over time. Multiple variants of SARS-CoV-2 have been documented in the United States and globally during this pandemic. Emergence of new variants is concerning.

Currently four variants of concern are: a) Alpha, known as B.1.1.7 and first identified in the United Kingdom; b) Beta, known as B.1.351 and first identified in South Africa; c) Gamma, also known as P1 and first identified in Japan and Brazil; and d) Delta, known as B.1.617.2 and first identified in India. Certain mutations in the virus spike gene region give the variants the ability to spread more quickly and possibly be less susceptible to neutralizing antibodies.[22,23,24] The Delta variant is more contagious than previous strains and is known to cause more than two times as many infections than the original COVID-19 strain.

The viral load due to Delta variant is 1,000 times higher in the infected individuals compared to earlier virus strains and was found to be recovered at a similar amount early in infection among both unvaccinated and fully vaccinated people.[38] Fully vaccinated people with Delta variant breakthrough infections can spread the virus to others, however, vaccinated people appear to spread the virus for a shorter time.[39] COVID-19 vaccines available for use in U.S. are still highly effective against Delta variant for prevention of severe infections and hospitalization.[39] The efficacy of the vaccines is reduced for general infection thus leading to breakthrough infections (20-25% in immunocompetent and 44% in immunocompromised). Therefore, fully vaccinated people should be tested 3-5 days after exposure with someone suspected or confirmed of having COVID-19, and to wear a mask in public indoor settings for 14 days or until they receive a negative test result.

The CDC is performing genomic surveillance with certain commercial, public health and academic laboratories, including ACLA member laboratories, to understand which variants are circulating more quickly and are important to characterize and track in terms of health. To date, in the US, there are no variants of high consequence with clear evidence that prevention measures or medical countermeasures have significantly reduced effectiveness.

FDA provided an update clarifying why new variants of the SARS-CoV-2 virus generally have low impact on molecular testing accuracy.[25] It is because most molecular tests rely on the detection of multiple regions of the genome and thus may be less impacted by genetic variation in the SARS-CoV-2 genome. Antigen and serological assays for SARS-CoV-2 are developed using the protein components of the virus, and therefore are also not at risk for reduced testing accuracy.

Conclusion

The COVID-19 pandemic is an ongoing public health threat that will continue for an extended time. A unique feature of SARS-CoV-2 is its ability to be transmitted while the host remains asymptomatic. Asymptomatic and pre-symptomatic transmission has been a critical factor in spread of the virus. The recent appearance of the Delta variant has added to risk of transmission in fully vaccinated people.

Surveillance programs that engage frequent testing using methods to detect the SARS-Cov-2 virus material identify active cases and isolate them for proper quarantine can help reduce transmission. Testing for viral presence with molecular amplification assays that detect SARS-CoV-2 nucleic acid performed in clinical laboratories (e.g., PCR, TMA, LAMP) should be prioritized for diagnosis of COVID-19 in symptomatic individuals.

Except to the extent authorized for over-the-counter home use, interpretation of test results requires oversight by a healthcare professional. Subsequent clinical action is best implemented with the advice of a healthcare professional engaged in a holistic testing program designed in partnership with a diagnostic laboratory.

To control viral transmission and quickly and accurately diagnose symptomatic hospitalized individuals, testing strategies may change over time as new tests and methods are developed or refined. Keeping abreast of such developments so that the right test is available to the right subject at the right time is imperative in our fight to control COVID-19.

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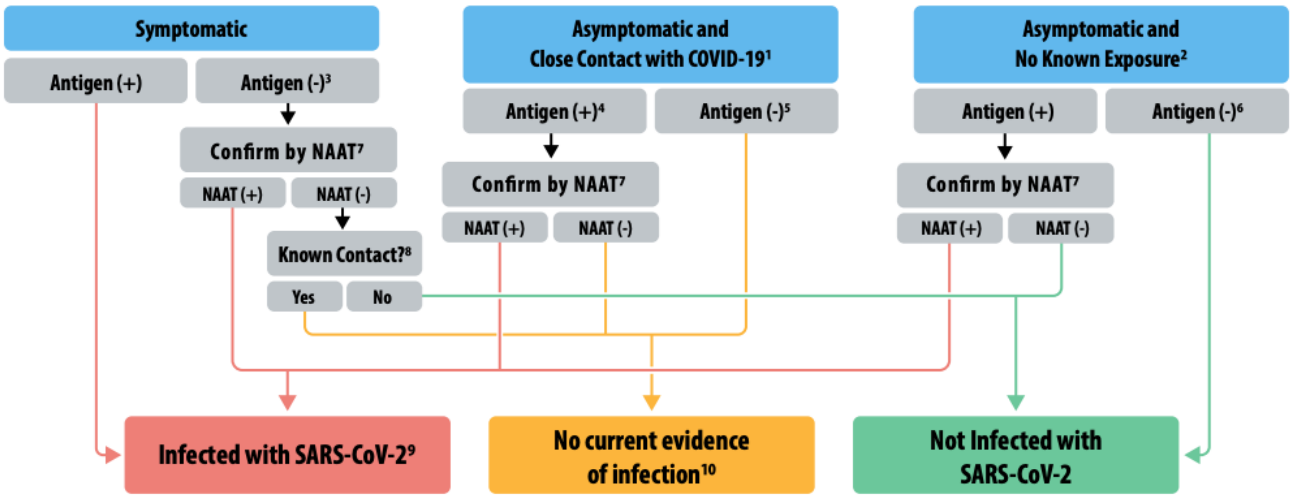
Fig 1: Antigen test algorithm



[1] Single, multiple, or continuous known exposure to a person with COVID-19 within the last 14 days; perform NAAT first if short turnaround time is available, if person cannot be effectively and safely quarantined, or if there are barriers to possible confirmatory testing.

[2] No known exposure to a person with COVID-19 within the last 14 days.

[3] If a symptomatic person has a low likelihood of SARS-CoV-2 infection, clinical discretion should determine if this negative antigen test result requires confirmatory testing.

[4] In instances of higher pretest probability, such as high incidence of incidence of infection in the community, clinical discretion should determine if this positive antigen result requires confirmation.

[5] In certain settings, serial antigen testing could be considered for those with a negative antigen test result; serial testing may not require confirmation of negative results. The role of a negative antigen test result in ending quarantine depends upon when it is performed in the quarantine period. See CDC's Options to Reduce Quarantine for guidance on use of antigen testing for this purpose and when a negative antigen test result indicates not infected with SARS-CoV-2.

[6] If prevalence of infection is not low in the community, clinical discretion should consider whether this negative antigen result requires confirmation.

[7] Nucleic acid amplification test; confirm within 48 hours using a NAAT, such as RT-PCR, that has been evaluated against FDA's reference panel for analytical sensitivity.

[8] Known exposure to a person with COVID-19 within the last 14 days; if unsure, clinical discretion should determine whether isolation is necessary.

[9] Isolation is necessary. See CDC's guidance for Isolation.

[10] Quarantine is necessary. See CDC's guidance for Quarantine; clinical discretion should determine if and when additional testing is necessary.

Fig 2: Testing algorithm for diagnosis of COVID-19 using molecular tests only