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

April 22, 2021

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, 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, NAAT 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 test results. 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.
- Easily incorporated in broad testing protocols, antibody tests may help optimize the utilization of molecular tests, enhancing the scalability of screening programs and reserving capacity of molecular tests for priority patients.
- Existing antibody tests, including those that predict neutralizing antibody titers, will play a role in determining if natural infection or vaccine administration led to a robust antibody response and determination of the durability of that response. High quality antibody tests can inform who should be prioritized for vaccine administration, should such an approach be needed due to limited vaccine supply. 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. Finally, this testing plays a key role in determining the presence of high titer antibodies to qualify donors for convalescent plasma, which may provide an alternative treatment option for hospitalized patients.

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. Approximately 350 million tests

to detect viral presence have been conducted with an overall positivity rate of about 8%.[9]

SARS-CoV-2 infections can be transmitted from asymptomatic as well as pre-symptomatic and symptomatic individuals.[1-4] Symptomatic individuals should isolate such that a delay in diagnosis does not lead to spread of the disease. In contrast, a delay in the diagnosis of asymptomatic or pre-symptomatic individuals who are infected can lead to community spread of the virus. 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 virus.

Assays for viral testing include those that detect SARS-CoV-2 nucleic acid and those that detect 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 processes. NAAT includes: 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 testing.

Commercial molecular assays have been in place since March 2020 for assistance in diagnosing active COVID-19 cases. In a symptomatic individual, an assay with higher analytic sensitivity (lower limit of detection) such as one of the tests categorized as NAAT enables a definitive clinical diagnosis. Among indiviudals infected with SARS-CoV-2, approximately 33% to 45% are asymptomatic; [3,4] therefore, we propose to continue using any of the 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 tests 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. Antigen tests can have clinical utility if NAAT tests are not readily available, especially if used on a frequent basis in congregate settings and are useful for screening persons on a frequent basis – such as the workplace and schools. Further, data demonstrate that the more frequently one is tested via antigen testing, the reliability of the testing increases. Those who do test positive using antigen tests should have confirmatory testing using a NAAT. Symptomatic individuals should isolate such that a diagnosis delay does not lead to further spread of the disease pending receipt of the NAAT test results. (Fig 1)

As noted, rapid POC antigen tests for SARS-CoV-2 can be a reliable first step to detect infection in asymptomatic individuals especially if followed by a NAAT to confirm a positive result[5]. Antigen negative, asymptomatic individuals are unlikely to be infectious, but a negative antigen test does not exclude being infectious at the time of antigen testing. Patients with a high pre-test probability of infection should have NAAT testing if antigen negative, but for screening of asymptomatic patients it is probably not necessary. The lower analytical sensitivity of antigen testing may be offset by more regular screening.[5] 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 spread and contain outbreaks.[9] POC assays backed up by appropriate NAAT testing 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 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. 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. PCR tests at the population level.[12] 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 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 test effectively 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.[13] 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

Based on the findings of these two 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.

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. High throughput antigen tests may be a better choice than POC antigen tests in such settings.

Role of Home-Based POC Testing

As of March 5, 2021, the US Food and Drug Administration (FDA) had granted an Emergency Use Authorization (EUA) for 38 home collection devices where after collection the sample is sent to the lab for testing. FDA has also provided EUAs for collection and testing options that can be performed at home, including one molecular test and two antigen tests.[14] Two of the tests require a physician prescription and are intended for symptomatic individuals, and the third test supports over-the-counter at-home testing in both symptomatic and asymptomatic individuals down to age 2 years.

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[15] 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 reveals a more complete picture of SARS-COV-2's spread and its changes over time. This testing provides 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[15] 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 PCR confirmation of SARS-CoV-2 infection.[16] Additionally, there is compelling evidence that using total antibody or combined IgG/IgM assays offers the highest sensitivity of detection.[17] 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% and 90.18%. 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%.[18]

Identifying individuals who may be able to donate COVID-19 convalescent plasma.[19]

Convalescent plasma is human plasma collected from individuals whose plasma contains anti-SARS-CoV-2 antibodies and who meet all donor eligibility requirements (21 CFR 630.10 and 21 CFR 630.15) and qualifications. On Feb 4, 2021, FDA issued a revision to the EUA for COVID-19 convalescent plasma for the treatment of hospitalized patients with COVID-19. In this update the authorization is limited for the use of only high titer COVID-19 convalescent plasma for the treatment of hospitalized patients with COVID-19 early in the disease course and to those hospitalized patients who have impaired humoral immunity and cannot produce an adequate antibody response. Plasma with low levels of antibodies has not been shown to be helpful in treating COVID-19. Plasma donations must be tested by registered or licensed blood establishments for anti-SARS-CoV-2 antibodies as a manufacturing step to determine suitability before release.

The EUA has also been updated to include several additional tests to be used in the manufacture of COVID-19 convalescent plasma. With this update, nine tests are now included in the EUA for testing plasma donations for anti-SARS-CoV-2 antibodies as a manufacturing step to determine suitability before release.

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 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 clinical trials to evaluate the efficacy of all vaccines under development. There may be a role for similar SARS-CoV-2 serological assays to determine the efficacy, durability, and the need for a booster dose.

Measurement of immunity or 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 immunity is not known at this time. Currently, the semi-quantitative assays are not interchangeable. Therefore, it is advised to use the same manufacturer's test to monitor immune response over a time. A universal calibrator for these assays has recently been made available and will therefore allow comparison of titers between assays. A positive neutralizing antibody test result means that a person is less likely to get a SARS-CoV-2 infection or significant COVID-19 illness.

Evaluation of persisting antibodies has been used to determine duration of vaccine-induced immunity in many viral infections. Qualitative detection of IgG antibodies correlates with clinical protection in measles, mumps, rubella, hepatitis B, varicella. For other viruses, quantitative detection of IgG is used to determine protection. To determine 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 performance of the serological testing has not been established in COVID-19 vaccinees.

Serology testing also plays a role in determining whether a person already has an immune response from a prior COVID-19 exposure. This can be useful pre-vaccination, in times and places where vaccine resources are scarce as part of a determination regarding who should be vaccinated. The use of post-vaccination antibody response as determined by serology testing, may help clinicians evaluate certain patients who may have some prior immune compromised condition, to determine whether their patient responded appropriately to a vaccination.

Duration of immunity to SARS-CoV-2

Many studies are ongoing to determine the duration of protective immunity to SARS-CoV-2 suggesting a level of protection that may last from months to a few years.[20] Investigators are looking at all components of the immune system including IgG antibodies, B and T cell responses. More studies are needed to make a conclusive decision on the duration of protective immunity in SARS-CoV-2, but early results are encouraging.

It is important to note that antibody levels represent one arm of the immune system and serve as a surrogate for other immune response mechanisms, including T-cell responses and other aspects of the immune system. Therefore, the reduction of 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 exposed to the virus again in the future, all arms of the immune system will be primed for a secondary immune response, which is often much more efficacious in clearing the virus than in a primary response. Recent publications show multi-fold reduction in NAAT positivity among people with positive SARS-CoV-2 antibody response compared to individuals with no antibodies. 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. [21] 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[22] 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. [23]

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 five variants of concern are: a) United Kingdom (UK), known as B.1.1.7; b) South Africa known as B.1.351; c) Brazil, known as P1 and d) two US-California, known as B.1.427 and B.1.429. These mutants carry approximately 21-23 total mutations of which about 8-10 are in the spike region. Certain mutations in the spike region give the variants the ability to spread more quickly and possibly be less susceptible to neutralizing antibodies.[24,25,26] The Centers for Disease Control (CDC) is performing nationwide genomic surveillance in collaboration with certain commercial 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.

FDA provided an update clarifying why new variants of the SARS-CoV-2 virus generally have low impact on molecular testing accuracy.[27] 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.

Authorized vaccines also remain effective in protecting the American public against currently circulating strains of COVID-19. Early experiments have shown that some of the neutralizing monoclonal antibodies against SARS-CoV-2 that have been authorized or are under development are less effective against some of the COVID-19 variants that have emerged. FDA is working with drug developers to accelerate the evaluation of new antibodies that could be effective against mutations.

Conclusion

The COVID-19 pandemic is an ongoing public health threat that will continue for some 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 worldwide spread of the virus. Surveillance programs that engage frequent testing using methods to detect the SARS-Cov-2 virus to identify active cases and isolate them for proper quarantine can help stop transmission. Testing for viral presence with 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, and acting on those results is best implemented with the advice of a healthcare professional engaged in a holistic testing program designed in partnership with a diagnostic laboratory

professional.

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.

References

1. Arons, Melissa., Kelly M Hatfield, Sujan C Reddy, Anne Kimball, Allison James, Jesica R Jacobs, Joanne Taylor, Kevin Spicer, Ana C Bardossy, Lisa P Oakley, et al. "Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility: NEJM." New England Journal of Medicine. May 28, 2020. Accessed April 02, 2021. https://www.nejm.org/doi/full/10.1056/NEJMoa2008457.
2. Sutton, Desmond, Karin Fuchs, Mary D'Alton, and Dena Goffman. "Universal Screening for SARS-CoV-2 in Women Admitted for Delivery." *New England Journal of Medicine* 382, no. 22 (2020): 2163-164. doi:10.1056/nejmc2009316.
3. Oran, Daniel P., and Eric J. Topol. "The Proportion of SARS-CoV-2 Infections That Are Asymptomatic: A Systematic Review." *Annals of Internal Medicine*. [Epub ahead of print 22 January 2021]. doi:10.7326/M20-6976
4. Frellick, Marcia. "Fauci: 'About 40%-45% of Infections Are Asymptomatic'." *Medscape*, Medscape, 18 Sept. 2020, www.medscape.com/viewarticle/937297.
5. "Interim Guidance for Antigen Testing for SARS-CoV-2." Centers for Disease Control and Prevention. Accessed April 02, 2021. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html.
6. Alexandersen, Soren, Anthony Chamings, and Tarka Raj Bhatta. "SARS-CoV-2 Genomic and Subgenomic RNAs in Diagnostic Samples Are Not an Indicator of Active Replication." 2020. doi:10.1101/2020.06.01.20119750.
7. Mina, Michael J., Roy Parker, and Daniel B. Larremore. "Rethinking Covid-19 Test Sensitivity — A Strategy for Containment." *New England Journal of Medicine* 383, no. 22 (2020). doi:10.1056/nejmp2025631.
8. Pekosz, A., Cooper, C. K., Parvu, V., Li, M., Andrews, J. C., Manabe, Y. C., . . . Roger-Dalbert, C. (2020). Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture. doi:10.1101/2020.10.02.20205708
9. "COVID Data Tracker." United States Laboratory Testing. Centers for Disease Control and Prevention. Accessed April 2, 2021. https://covid.cdc.gov/covid-data-tracker/#testing_testsperformed.
10. "In Vitro Diagnostics EUAs." Emergency Use Authorizations for Medical Devices. U.S. Food and Drug Administration. Center for Devices and Radiological Health. Accessed April 2, 2021. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas#individual-antigen.
11. "In Vitro Diagnostics EUAs." Emergency Use Authorizations for Medical Devices. U.S. Food and Drug Administration. Center for Devices and Radiological Health. Accessed April 2, 2021. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas#individual-antigen.
12. Larremore, Daniel B., Bryan Wilder, Evan Lester, Soraya Shehata, James M. Burke, James A. Hay, Milind Tambe, Michael J. Mina, and Roy Parker. "Test Sensitivity Is Secondary to Frequency and Turnaround Time for COVID-19 Surveillance." 2020. doi:10.1101/2020.06.22.20136309.
13. Paltiel, A. David, Amy Zheng, and Rochelle P. Walensky. "Assessment of SARS-CoV-2 Screening Strategies to Permit the Safe Reopening of College Campuses in the United States." *JAMA Network Open* 3, no. 7 (2020). doi:10.1001/jamanetworkopen.2020.16818.
14. "COVID-19 Update." Press Announcements . U.S. Food and Drug Administration. Accessed April 2, 2021. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-march-5-2021.
15. "Interim Guidelines for COVID-19 Antibody Testing." Resources for Labs. Centers for Disease Control and Prevention. Accessed April 2, 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html#anchor_1590264293982.
16. Wu, Fan, Aojie Wang, Mei Liu, Qimin Wang, Jun Chen, Shuai Xia, Yun Ling, Yuling Zhang, Jingna Xun, Lu Lu, Shibo Jiang, Hongzhou Lu, Yumei Wen, and Jinghe Huang. "Neutralizing Antibody Responses to

SARS-CoV-2 in a COVID-19 Recovered Patient Cohort and Their Implications." 2020. doi:10.1101/2020.03.30.20047365.
17. Espejo, Andrea P., Yamac Akgun, Abdulaziz F Al Mana, Youley Tjendra, Nicolas C. Millan, Carmen Gomez-Fernandez, and Carolyn Cray. "Review of Current Advances in Serologic Testing for COVID-19." *American Journal of Clinical Pathology* 154, no. 3 (2020): 293-304. doi:10.1093/ajcp/aqaa112.
18. Yangchun, Feng. "Optimize Clinical Laboratory Diagnosis of COVID-19 from Suspect Cases by Likelihood Ratio of SARS-CoV-2 IgM and IgG Antibody." 2020. doi:10.1101/2020.04.07.20053660.
19. "FDA Updates Emergency Use Authorization for COVID-19 Convalescent Plasma to Reflect New Data." FDA News. Center for Devices and Radiological Health. U.S. Food and Drug Administration. Accessed April 2, 2021. https://www.fda.gov/news-events/fda-brief/fda-brief-fda-updates-emergency-use-authorization-covid-19-convalescent-plasma-reflect-new-data.
20. Dan, Jennifer M., Jose Mateus, and Yu Kato. "Immunological Memory to SARS-CoV-2 Assessed for Greater than Six Months after Infection: NCRC." 2019 Novel Coronavirus Research Compendium (NCRC). December 06, 2020. Accessed April 02, 2021. https://ncrc.jhsph.edu/research/immunological-memory-to-sars-cov-2-assessed-for-greater-than-six-months-after-infection/.
21. Harvey, Raymond A., Jeremy A. Rassen, Carly A. Kabelac, et. al. "Association of SARS-CoV-2 Seropositive Antibody Test With Risk of Future Infection." *JAMA Internal Medicine*, 2021. doi:10.1001/jamainternmed.2021.0366.
22. Lumley, Sheila F., et al. "Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers: NEJM." New England Journal of Medicine. March 23, 2021. Accessed April 02, 2021. https://www.nejm.org/doi/full/10.1056/NEJMoa2034545.
23. Dimeglio, Chloé, Fabrice Herin, Marcel Miedougé, Guillaume Martin-Blondel, Jean-Marc Soulat, and Jacques Izopet. "Protection of Healthcare Workers Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Reinfection." *Clinical Infectious Diseases*, 2021. doi:10.1093/cid/ciab069.
24. Zhou, Bin, Tran Thi Nhu Thao, Donata Hoffmann, et. al. "SARS-CoV-2 Spike D614G Variant Confers Enhanced Replication and Transmissibility." *bioRxiv* 2020. doi:10.1101/2020.10.27.357558.
25. Volz, Erik, Verity Hill, John T. Mccrone, et. al. "Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on Transmissibility and Pathogenicity." *Cell 2021*, no. 182 (2020): 812-17. doi:10.1101/2020.07.31.20166082.
26. Korber B, Fischer WM, Gnanakaran S, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell 2021; 182(812-7). doi: https://doi.org/10.1016/j.cell.2020.06.043external icon.
27. "FDA Continues Important Work to Support Medical Product Development to Address New Virus Variants." FDA News. Center for Devices and Radiological Health. U.S. Food and Drug Administration. April 1, 2021. www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-continues-important-work-support-medical-product-development-address.

Fig 1: Testing algorithm for diagnosis of COVID-19 using molecular tests only

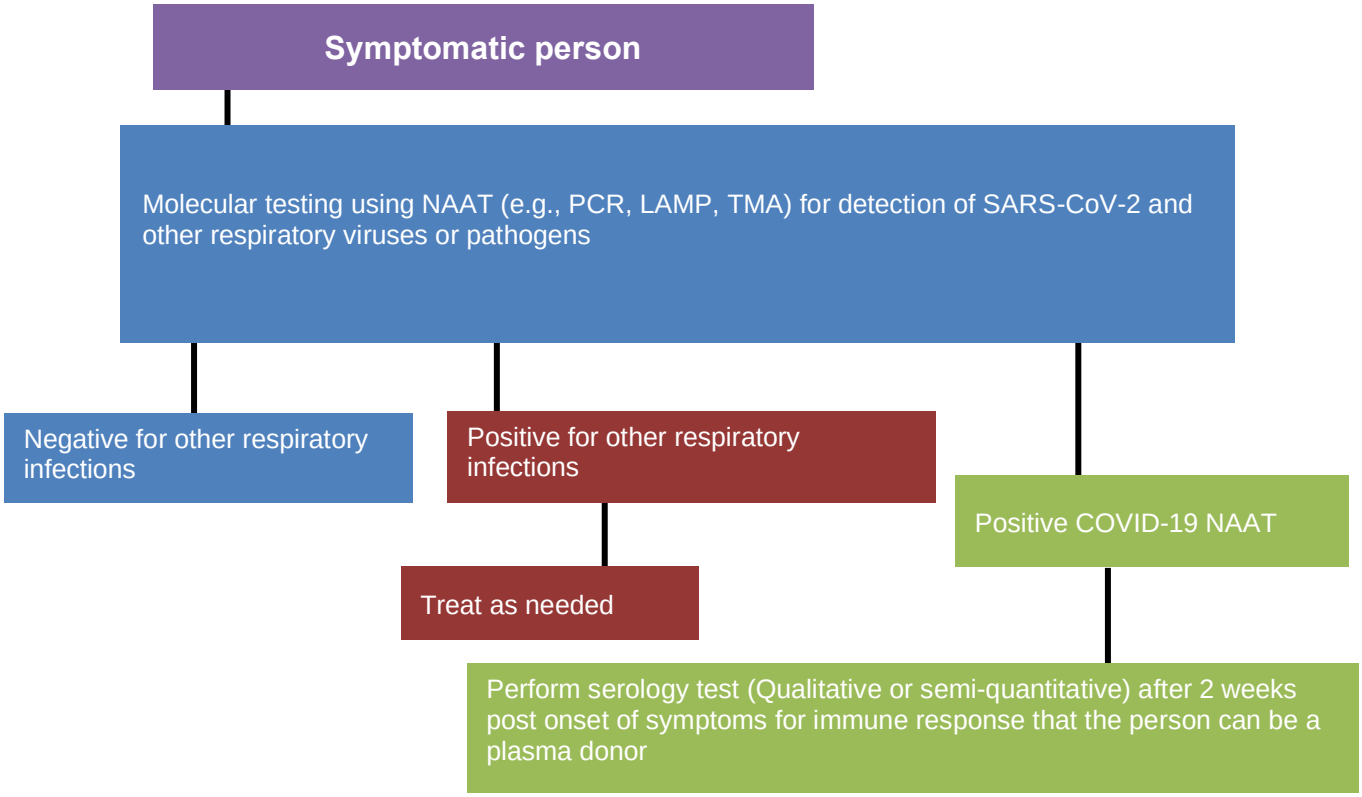

Fig 2: Testing algorithm for screening and surveillance of asymptomatic and pre-symptomatic individuals using rapid point of care **(POC) antigen test**