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Corporate Medical Policy

Coronavirus Testing in the Outpatient Setting AHS – G2174

File Name: coronavirus_testing_in_ the_outpatient_setting
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Description of Procedure or Service

Human coronaviruses, first characterized in the 1960s, are named based on the spiked proteins located on their surface. As of 2020, seven coronaviruses are known to infect humans. Four, of which—229E, NL63, OC43, and HKU1—are associated with the common cold. MERS-CoV is the coronavirus that causes Middle East Respiratory Syndrome, or MERS. SARS-CoV is the causative agent of Severe Acute Respiratory Syndrome (SARS), and SARS-CoV-2 is the virus that causes coronavirus disease 2019, or COVID-19 (CDC, 2020b). As of November 11, 2022, the United States had reported more than 634,709,332 confirmed cases of COVID-19 and over 6,609,119 reported COVID-19 deaths (JHU, 2022). Testing for a possible coronavirus infection can include molecular tests, such as nucleic acid-based testing like reverse transcription polymerase chain reaction (RT-PCR); host antibody testing; and antigen testing.

Related Policies:

Fecal Analysis in the Diagnosis of Intestinal Dysbiosis AHS-G2060

Pathogen Panel Testing AHS-G2149

Identification of Microorganisms Using Nucleic Acid Probes AHS-M2097

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for coronavirus testing in the outpatient setting when it is determined to be medically necessary because the medical criteria and guidelines shown below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

This policy only addresses testing for the purpose of medical decision making in the outpatient setting. This policy does not address work, school, state, or federally mandated SARS-CoV-2 testing

When Coronavirus Testing in the Outpatient Setting is covered

- Reimbursement is allowed for targeted nucleic acid testing (e.g., RT-PCR, rapid molecular tests) for COVID-19 (SARS-CoV-2), in any of the following situations:
    - For individuals displaying signs and symptoms of possible COVID-19 infection (**See Note 1**).
    - For asymptomatic individuals with known exposure to COVID-19, EXCEPT when the individual has had a previous COVID-19 infection within the last 90 days.

- Reimbursement for individuals with signs or symptoms of SARS **and** who have traveled to endemic areas or who have been exposed to persons with SARS, targeted nucleic acid testing (e.g., RT-PCR) for detection of severe acute respiratory syndrome (SARS) coronavirus RNA is allowed.

- Reimbursement for individuals with signs and symptoms of Middle East respiratory syndrome (MERS) **and** who have traveled to endemic areas or who have been exposed to persons with MERS, targeted nucleic acid testing, such as RT-PCR, for the detection of MERS coronavirus RNA is allowed.

- Reimbursement is allowed for host antibody serology testing to support a diagnosis of multisystem inflammatory syndrome in children (MIS-C) (**see Note 2**), multisystem inflammatory syndrome in adults (MIS-A) (**see Note 3**), or post-acute sequelae of SARS-CoV-2 infection (PASC).

- Reimbursement is allowed for antigen-detecting diagnostic tests for SARS-CoV-2 (e.g., antigen rapid tests) for symptomatic individuals.

- Reimbursement for individuals with signs and symptoms of a respiratory tract infection (**see Note 4**), multiplex PCR-based panel testing of up to 5 respiratory pathogens is allowed.

- Reimbursement for individuals with signs and symptoms of a respiratory tract infection (**see Note 4**), antigen panel testing of up to 5 antigens is allowed.

Note 1: Signs and symptoms associated with a possible COVID-19 infection can include a fever, cough, fatigue, shortness of breath or difficulty breathing, congestion or runny nose, chills, muscle pain, headache, sore throat, new loss of taste or smell, nausea, vomiting, diarrhea, conjunctivitis, rash on skin or discoloration of fingers or toes (CDC, 2020e; WHO, 2023).

Note 2: According to the CDC, evidence of possible MIS-C includes (CDC, 2021c):

- Fever of at least 38.0˚C for at least 24 hours
- Multisystem (2 or more) organ involvement
- Laboratory evidence of inflammation, "including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin (CDC, 2020d)"

Some children may fulfill full or partial criteria for Kawasaki disease

Note 3: According to the CDC, evidence of possible MIS-A includes (Morris et al., 2020; Patel et al., 2021):

- a severe illness requiring hospitalization in a person aged ≥21 years;
- a positive test result for current or previous SARS-CoV-2 infection (nucleic acid, antigen, or antibody) during admission or in the previous 12 weeks;

- severe dysfunction of one or more extrapulmonary organ systems (e.g., hypotension or shock, cardiac dysfunction, arterial or venous thrombosis or thromboembolism, or acute liver injury);
- laboratory evidence of severe inflammation (e.g., elevated CRP, ferritin, D-dimer, or interleukin-6);
- absence of severe respiratory illness (to exclude patients in which inflammation and organ dysfunction might be attributable simply to tissue hypoxia).

Note 4: Signs and symptoms of a respiratory tract infection:

- A temperature greater than 102°F
- Pronounced dyspnea,
- Tachypnea, or
- Tachycardia.

When Coronavirus Testing in the Outpatient Setting is not covered

1. Reimbursement for the diagnosis of SARS-CoV-2 reinfection, whole genome sequencing of paired specimens from distinct lineages (as defined in Nextstrain or GISAID) is not allowed.

2. Reimbursement is not allowed for antigen panel testing of 6 or more antigens.

3. Reimbursement is not allowed for multiplex PCR-based panel testing of 6 or more respiratory pathogens

4. Reimbursement is not allowed for host antibody serology testing for all other situations not described above.

5. Reimbursement is not allowed for SARS-CoV-2 genotyping in the outpatient setting.

6. Reimbursement is not allowed for neutralization antibody testing for SARS-CoV-2 for any indications.

7. Reimbursement is not allowed for testing for other endemic coronaviruses, such as 229E, NL63, OC43, and HKU1

AMA standard practice for COVID-19 testing states not to include both the HCPCS and AMA code for the same procedure on the same DOS and that only one code should be used, therefore only one code per date of service will be reimbursed.

Specimen collection codes for coronavirus testing are considered incidental and will not be reimbursed.

Policy Guidelines

On March 11, 2020, the World Health Organization (WHO) declared the novel coronavirus SARS-CoV-2, or COVID-19, a global pandemic (Cucinotta & Vanelli, 2020). COVID-19 is the third recent human coronavirus to be declared an emergency. SARS (Severe Acute Respiratory Syndrome) was recognized as an emergency by the WHO in February 2003 (WHO, 2022c). This outbreak in 2003 resulted in over 8000 cases in 26 different countries. Since 2003, only four limited reoccurrences have been reported according to the WHO—three incidences are due to laboratory accidents (in Taipei and Singapore) and one incident of undetermined source in China (WHO, 2022c). As early as September 2012, another human coronavirus, MERS-CoV, began to spread in the Middle East, causing Middle East Respiratory Syndrome (MERS). Although the WHO did not initially declare MERS an emergency, they have since

added MERS to their list of pandemic/epidemic diseases. Since September 2012 and as of the end of October 2021, the WHO reports 2574 laboratory-confirmed cases of MERS with 858 MERS-associated deaths (34.4% fatality rate) in 27 countries (WHO, 2022b).

Unlike the initial SARS and MERS outbreaks that were predominantly regionally contained, COVID-19 became a global pandemic. According to the WHO, as of November 11,2022, there were more than 630 million confirmed cases of COVID-19 with over 6,584,104 confirmed deaths worldwide (WHO, 2022a). Infection from the novel human coronavirus SARS-CoV-2 can result in coronavirus disease 2019 (COVID-19). The WHO reports approximately 15% of individuals with COVID-19 develop severe disease requiring oxygen support while 5% develop "critical disease" with complications such as respiratory failure or multiorgan failure (WHO, 2021b). Older individuals and patients with comorbidities—such as cardiovascular disease, diabetes mellitus, hypertension, chronic lung disease, cancer, chronic kidney disease, obesity, and smoking—have an increased likelihood of poor outcomes (McIntosh, 2023). Sepsis, multiorgan failure (including the kidney, liver, and heart), pneumonia, and acute respiratory distress syndrome (ARDS) can also occur (WHO, 2021b; Yang et al., 2020). Severe outcomes have been associated with the following laboratory features: lymphopenia, elevated liver enzymes, elevated lactate dehydrogenase (LDH), elevated inflammatory markers (such as CRP and ferritin), elevated D-dimer, elevated prothrombin time (PT), elevated troponin, elevated creatine phosphokinase (CPK), and acute kidney injury (McIntosh, 2023).

Much of what has generated this global pandemic is attributed to the different levels of transmissibility of the SARS-CoV-2 virus compared to SARS-CoV-1 and MERS, which can arise from the viral load. Simply put, viral load is the number of viral particles/virions in a milliliter of blood (Ryding, 2020). The viral load of SARS-CoV-2 "peaks around the time of symptom onset, followed by a gradual decrease to a low level after about 10 days. Regarding the period of high infectiousness, a recent study reported that exposure to an index case within 5 days of symptom onset confers a high risk of secondary transmission" (Kawasuji et al., 2020). This finding was corroborated by other studies, which found that "SARS-CoV-2 viral load in the upper respiratory tract appeared to peak in the first week of illness, whereas that of SARS-CoV peaked at days 10–14 and that of MERS-CoV peaked at days 7–10;" because SARS-CoV-2 viral load peaks faster, it can be more transmissible earlier in the disease course (Cevik et al., 2021). However, after reaching its peak during symptom onset, the viral load decreases "monotonically" (Kawasuji et al., 2020). If viral loads do not decrease, patients will be more likely to suffer worse outcomes and require hospitalization (Griffin, 2020). Viral load has been found to be either similar among symptomatic and asymptomatic COVID-19 positive individuals, or higher among symptomatic individuals (Kawasuji et al., 2020; Nackerdien, 2020). Infectiousness of COVID-19 also correlates with shedding, meaning that the viral particles can replicate in an individual and spread in the environment to others. The mean duration of SARS-CoV-2 RNA shedding "was 17.0 days (95% CI 15·5–18·6; 43 studies, 3229 individuals) in upper respiratory tract, 14.6 days (9·3–20·0; seven studies, 260 individuals) in lower respiratory tract, 17.2 days (14·4–20·1; 13 studies, 586 individuals) in stool, and 16.6 days (3·6–29·7; two studies, 108 individuals) in serum samples," with maximum shedding duration reaching "83 days in the upper respiratory tract, 59 days in the lower respiratory tract, 126 days in stools, and 60 days in serum"(Cevik et al., 2021).

In children and adolescents, reports of a multisystem inflammatory syndrome (MIS-C) with similarities to Kawasaki disease and toxic shock syndrome have been linked to COVID-19 (CDC, 2020d; DeBiasi et al., 2020; Jones et al., 2020; Verdoni et al., 2020; WHO, 2020c). Multisystem inflammatory syndrome has also been reported in adults (MIS-A). From June to October 2020, researchers reported 27 cases of MIS-A in the US and UK (Baum, 2020). The case definition of MIS-A includes "(1) hospitalization without evidence of severe respiratory illness (to exclude hypoxia as the cause of the signs and symptoms), (2) extrapulmonary organ system involvement (including hypotension or shock, cardiac dysfunction, arterial or venous thromboembolism, acute liver injury, or dermatologic abnormalities), and (3) laboratory evidence of acute inflammation (e.g., highly elevated C-reactive protein, ferritin, D-dimer, or interleukin-6)" (Baum, 2020). Most patients present with a fever >100.4 °F, cardiac abnormalities (arrhythmias, elevated troponin levels, or left or right ventricular dysfunction), and gastrointestinal

symptoms. Rare symptoms include dermatological manifestations or respiratory symptoms such as pleural effusion. Patients may have elevated laboratory markers of inflammation including CRP, ferritin, and markers of coagulopathy including D-dimer (Morris et al., 2020).

As SARS-CoV-2 has continuously mutated over the course of the pandemic, CDC has adjusted their categorizations of the numerous variants based on shared attributes that may require public action and on available information. CDC lists four variant classifications on their website: variants being monitored (VBM), variants of interest (VOI), variants of concern (VOC), and variants of high consequence (VOHC). Variants being monitored (VBMs) are described as "lineages whose data indicates there is a potential or clear impact on available medical countermeasures", "lineages that cause more severe disease or increased transmission but are no longer detected", or "lineages previously designated as a VOI, VOC, or VOHC that are currently circulating at very low levels, in the United States". As such, VBMs "do not pose a significant and imminent risk to public health in the United States" and VOIs and VOCs may be downgraded to this list when evidence suggests that they no longer pose significant risk to public health (CDC, 2023c). The list of possible attributes for variants of interest (VOIs) include the presence of "specific genetic markers that are predicted to affect transmission, diagnostics, therapeutics, or immune escape", "evidence that it is the cause of an increased proportion of cases or unique outbreak clusters", and "limited prevalence or expansion in the US or in other countries". In addition to including possible features of VOIs, variants of concern (VOCs) are marked by a "increase in transmissibility", "more severe disease (for example, increased hospitalizations or deaths)", "significant reduction in neutralization by antibodies generated during previous infection or vaccination", and "reduced effectiveness of treatments or vaccines, or diagnostic detection failures". A variant of high consequence (VOHC) "has clear evidence that prevention measures or medical countermeasures (MCMs) have significantly reduced effectiveness relative to previously circulating variants" (CDC, 2023c). Currently, all the variants being monitored by CDC fall in VBM status except for the Omicron strain (B.1.1.529 and descendant lineages), which is labeled a VOC.

CDC indicates three vaccines as authorized and recommended to prevent COVID-19 in the US: Pfizer-BioNTech COVID-19 Vaccine, Bivalent; Moderna COVID-19 Vaccine, Bivalent; and Novavax COVID-19 Vaccine, Adjuvanted. The Pfizer-BioNTech and Moderna COVID-19 vaccines are mRNA vaccines, which instruct B and T lymphocytes to fight off that specific mRNA-encoded protein from COVID-19 in the event of future exposure. Novavax is a protein subunit vaccine that delivers pieces (spike proteins) of the virus that causes COVID-19, as well as an adjuvant that helps the immune system respond in the event of future exposure (CDC, 2023a).

Besides the viruses associated with SARS, MERS, and COVID-19, four other human coronaviruses (HCoVs) are currently known—229E, NL63, OC43, and HKU1. These four viruses are considered endemic to the human population, and they typically cause mild respiratory tract infections associated with the common cold; in fact, it is approximated that up to one-third of all "common colds" may be due to one of these four endemic human coronaviruses. These HCoVs can cause both upper and lower respiratory infections, but they typically result in relatively mild, or even asymptomatic, cases. In immunosuppressed individuals, including those with pre-existing pulmonary diseases, progression to acute respiratory failure can occur in some cases (Corman et al., 2019; Ludwig & Zarbock, 2020).

Nucleic Acid Testing for Human Coronavirus Infections

Coronaviruses are a family of enveloped, single-stranded positive-sense RNA viruses. During the initial phase of infection, the virus can be detected in respiratory specimen due to high concentrations of viral RNA **(Figure 1).** RT-PCR is a powerful molecular technique that synthesizes complimentary DNA (cDNA) from the initial RNA template and uses primers to manufacture multiple cDNA copies for analysis. RT-PCR, when used with appropriate primers targeting the SARS-CoV-2 RNA, is used to diagnose an acute infection. The CDC RT-PCR Diagnostic Panel detects SARS-CoV-2 virus in the upper and lower respiratory specimen. The CDC has released standard primers to detect SARS-CoV-2 RNA, but any primers or probes that receive an Emergency Use Authorization (EUA) label may also be used

with the CDC's RT-PCR Diagnostic Panel (CDC, 2022a). As depicted in **Figure 1**, the concentration of viral RNA decreases as the immune system fights the infection, and very low or undetectable viral RNA levels are typically present after an individual has recovered. Consequently, RT-PCR cannot be used to screen for a past infection. Another limitation to RT-PCR is that it does require specific instrumentation, and, therefore, is less amenable as a rapid, point-of-care test. RT-PCR results of SARS-CoV-2 may fluctuate and become unstable over time, thus requiring other clinical diagnostic measures, such as computerized tomography imaging to supplement isolation, discharge, and any transfers during this epi demic (Li et al., 2020).

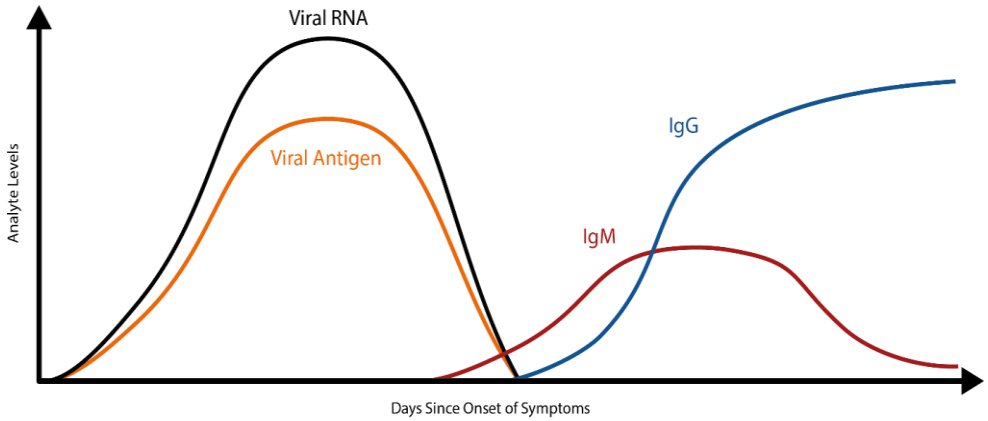

Figure 1: *General time course of a viral infection, such as SARS-CoV-2. This is for illustrative purposes and should not be used as a primary reference or for diagnostic purposes. The original content can be found within the references*

Clinical Utility and Validity of Nucleic Acid Testing

Many studies have been performed to date to evaluate the analytical performance of RT-PCR. One study, using a high-throughput platform, for example, reported a limit of detection (LoD) of 689.3 copies/mL and 275.72 copies per reaction at 95% detection probability (Pfefferle et al., 2020). The WHO diagnostic RT-PCR test utilizes two genes--the *E* gene as the molecular target (where the limit is 3.9 copies per reaction) and the *RdRp* gene as the molecular target (limit of 3.6 copies per reaction) (Lippi et al., 2020). One recent study reported possible *in vitro* cross-reactivity between the RdRp-based method used predominantly in European labs with SARS-CoV in cell culture (Chan et al., 2020). SARS-CoV is the coronavirus that caused the initial SARS (Severe Acute Respiratory Syndrome) outbreak in 2003 (WHO, 2022c). The likelihood of either a co-infection of SARS-CoV and SARS-CoV-2 or a concurrent outbreak of both viruses is small. The CDC diagnostic panel test does not target the *RdRp* gene; it consists of two primer/probe sets of the *N* gene and one primer/probe set for human RNase P gene (*RP*) as the control. The CDC diagnostic panel has a reported limit of 1.0 – 3.2 copies/µL (CDC, 2020a; Lippi et al., 2020). Reports of initial negative RT-PCR results in individuals who later develop symptomatic COVID-19 have been published, but this may occur if the sample was not properly collected or if it was taken from the patient early in the infection during the initial incubation period of SARS-CoV-2, which is approximately 6 days (interquartile range [IQR], 2 – 11 days) (Backer et al., 2020; Lippi et al., 2020). Consequently, it is important to remember that "Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information (LabCorp, 2020b)."

To compare and analyze the diagnostic efficacy of two RT-PCR test kits for detection of SARS-CoV-2, Lu et al. (2020) studied throat swab samples from 18 hospitalized patients with a clinical COVID-19 diagnosis and 100 hospitalized patients without COVID-19 diagnosis. Two different RT-PCR tests from Sansure Biotech Inc (SansureBiotech, 2020) and Shanghai BioGerm Biotechnology Co., Ltd (BioGerm, 2020) were used. The table below (Lu et al., 2020) shows that the detection efficacy of BioGerm PCR kit was higher than that of the Sansure PCR kit. These two kits had the same specificity and positive predictive value, but the sensitivity of the Sansure PCR kit was 83.3%, whereas the sensitivity of the BioGerm PCR kit was 94.4%. For the Sansure PCR kit, 3 of the 18 samples were false-negative results, and for the BioGerm PCR kit, 1 of the 18 samples was a false-negative result. No false-positive results were detected in these tests. The author suggests that "these findings provide important information for the ongoing optimization of viral detection assays following the emergence of COVID-19" (Lu et al., 2020).

Table 2. *Diagnosis efficacy of Sansure and BioGerm test kits for SARS-CoV-2 nucleic acid detection*

	COVID-19 samples (n = 18)		None- COVID-19 samples (n = 100)		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Kappa (95%CI)
Test kits	Positive	Negative	Positive	Negative					
Sansure	15	3	0	100	0.833(0.577-0.956)	1.000(0.954-1.000)	1.000(0.747-1.000)	0.971(0.911-0.992)	0.894(0.726-1.000)
BioGerm	17	1	0	100	0.944(0.706-0.997)	1.000(0.954-1.000)	1.000(0.771-1.000)	0.990(0.938-0.999)	0.966(0.880-1.000)

In a case series study of multisystem inflammatory syndrome in adults (MIS-A) associated with SARS-CoV-2 infection, 16 patients ranging from 21 to 50 years old were enrolled and tested with PCR assay. 10 out of 16 patients had positive SARS-CoV-2 PCR test results at the time of admission. Two patients had positive SARS-CoV-2 PCR test results 14 and 37 days before admission and negative PCR results at the time of admission. Three patients had positive SARS-CoV-2 PCR test results 25–41 days before admission and continued positive PCR test results at the time of admission. "Given the high proportion of MIS-C patients with negative PCR testing, clinical guidelines recommend the use of both antibody and viral testing to assist with diagnosis" (Morris et al., 2020).

Li et al. (2021) conducted a cross-sectional analysis on 30 patients with COVID-19 diagnoses to compare the sensitivity of SARS-CoV-2 testing in anterior nasal vestibular swabs versus oropharyngeal swabs. After specimen collection, RT-PCR assays were used to test them for SARS-CoV-2. They found that 56.7% of the patients tested positive using oropharyngeal specimen, whereas 66.7% of patients tested positive with the nasal swab specimens. Ultimately, there is "adequate sensitivity" to use the less invasive anterior nasal vestibular swabs to detect COVID-19 infection confirmed by RT-PCR (Li et al., 2021).

Yau et al. (2021) evaluated the clinical utility of a rapid "on-demand" PCR-based testing service in an acute hospital setting. In an effort to increase hospital efficiency starting from July 2020, the researchers

focused on moving patients quickly to isolation rooms and minimize potential risk of transmission in crowded areas. From their study, it was found that the "daily/monthly PCR positive test numbers approximately followed the local and national UK trend in COVID-19 case numbers, with the daily case numbers being reflective of the Nov and Dec 2020 surges." It ultimately helped to reduce "unnecessary 'length-of-stay' in a busy acute respiratory ward." Patients were able to be rapidly separated based on COVID-19 positive diagnosis and the system in place reduced exposure and nosocomial transmission (Yau et al., 2021).

Dighe studied a lateral flow strip-based RNA extraction and amplification-free nucleic acid test (NAT) for rapid diagnosis of COVID-19 at point of care which takes no longer than 30 minutes. This test uses highly specific 6-carboxyfluorescein (6-FAM) and biotin labeled antisense oligonucleotides (ASOs) as probes those are designed to target the N-gene sequence of COVID-19. This study evaluated 60 samples using the lateral flow assay and results were compared with the FDA-approved TaqPath RT-PCR kit. According to the results, the assay obtained almost 99.99% accuracy and specificity. The authors conclude that this new LFA method could be "expanded beyond COVID-19 detection, simply by altering its targeting antisense oligonucleotides, to become a global health technology that contributes to providing low-cost diagnostics" (Dighe et al., 2022).

Mawhorter et al. (2022) investigated the impact and cost of a routine pre-operative COVID-19 PCR testing algorithm for asymptomatic patients before elective surgery at a rural academic institution per recommendations by the American College of Surgeons. From 7579 pre-procedural tests that were completed since May 2020 using the protocol, the study yielded 31 (0.41%) positive results in asymptomatic patients. With these positive results, there were impacts on both the cost and delay of the procedure. The results showed that "20 procedures (62.5%) were delayed an average of 49 days, 8 were not performed, and 3 proceeded without delay," with a prolonged delay for the 3 urological procedures of 59 days. They also identified that the number needed to test for one positive result was 244, with $11,573 as cost for each positive result. This analysis found that the hospital was able to be more cost-effective (each test was $34-54) with a standardized testing algorithm prior to procedure performance (Mawhorter et al., 2022).

Host Antibody Testing

The COVID-19 illness begins with an initial infection by SARS-CoV-2. Viral invasion stimulates the host immune response to produce immunoglobulins, such as IgM, IgA, and IgG, that can target the invading virus. However, there is a delay between the time of initial infection and the production of immunoglobulins **(Figure 1)** (The_Native_Antigen_Company, 2020). Typically, several days after the initial onset of symptoms, the first IgM immunoglobulins are produced to combat the viral infection. IgA (not shown in Figure 1), immunoglobulins secreted to protect predominantly the mucosal linings of the gastrointestinal, respiratory, and genitourinary tracts (Woof & Kerr, 2006), typically have a half-life of 4 – 6 days (Morell et al., 1973). Finally, IgG, the long-term immunoglobulins found within body fluids that fight bacterial and viral infections, are produced and IgM production wanes. Some limited studies have indicated that some individuals may initially produce IgM and IgG antibodies concurrently, but additional research is needed (Padoan et al., 2020).

Serological host antibody tests can detect the presence of IgM and IgG antibodies that an individual has developed in response to an infection—in this case, a SARS-CoV-2 viral infection. The test may report total antibodies present, meaning either it does not distinguish between IgG and IgM or that it is reporting the sum of IgG and IgM. This is sometimes referred to as "total antibody testing". On the other hand, the test may be specific for one antibody, such as IgG or IgM, or the test may claim to accurately distinguish between the antibodies.

Another type of antibody testing is "neutralizing" antibody detection, as opposed to "binding" antibody detection described above. This process involves incubating serum with a live version of the virus. The analytes of interest are the antibodies that have the capability to prevent infection by the virus (i.e.

neutralization). Identification of these antibodies may contain useful clinical information and are often reported in an aggregate titer, as opposed to specifying each individual antibody (CDC, 2022e; Espejo et al., 2020). Due to use of live virus, these tests may need to be performed in a higher biosafety laboratory, although some techniques using pseudoviruses may be performed in less restrictive laboratories (CDC, 2022f).

Clinical Utility and Validity of Host Antibody Testing

Antibody testing has many potential uses. Ideally, the use of an accurate, reliable antibody test could possibly show whether someone has previously been exposed to the virus. This could indicate possible immunity in an individual. Please note that ***the antibody test is not used as a diagnostic test, meaning it should not be used to diagnose an acute infection***. Within the FDA policy for diagnostic testing for COVID-19, issued on November 15, 2021 they state, "Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infection" (FDA, 2022e).

The FDA published a Frequently Asked Questions (FAQ) update on March 19, 2021. In it, they remarked that antibody tests cannot be used to diagnose COVID-19, and that molecular or antigen tests are available for diagnostic purposes (FDA, 2022a).

Since SARS-CoV-2 is a new, emerging virus, it is not known for certain how long it takes for the seroconversion to occur or when antibodies start to appear in the blood at high enough concentrations for accurate testing results. A recent study published in *Clinical Infectious Diseases* reports an average of seroconversion time for IgM and IgG at 12 and 14 days, respectively (Zhao et al., 2020). A small study (n=34 patients) reports the presence of IgG for at least seven weeks (the duration of the study) (Xiao et al., 2020). Another study, however, reports that IgM testing has similar, if not better positive detection rate than PCR 5.5 days after initial onset of symptoms; however, the total window of antibody detection for IgM was only 5 days long (Guo et al., 2020) **(See Figure 1)**. If the patient was not tested during the detection window, then the individual would not necessarily have a "positive" result for IgM. The authors also report the detection of IgA antibodies (median onset at 5 days after initial symptoms [IQR 3 – 6 days]), and 92.7% of total samples report a positive result for IgA. This same study also reports that IgG detection occurs, on average, fourteen days after initial onset of symptoms (Guo et al., 2020). Another study reports that IgA-based ELISA testing has higher sensitivity than IgG-based ELISA testing, but the IgG-based ELISA testing has higher specificity. The authors recommend IgG-based testing over the IgA-based testing in immunosurveillance studies since IgG has a longer biological half-life (Okba et al., 2020). At least one published study to date has reported that as many as 6.9% of individuals who previously had tested positive with RT-PCR results did not show the presence of antibodies for the length of the study (at least 40 days after the initial onset of symptoms) (Zhao et al., 2020).

Ideally, any rapid diagnostic test for the outpatient setting must be accurate and reliable. Current research indicates that the diagnostic window for IgA and IgM is very limited. Some data indicate that host antibody testing can also yield inaccuracies. Also, for IgG testing, the significance of positive results is questionable at the current time. A positive result could indicate a previous infection, assuming the test did not cross-react with any other IgG the host produced in response to one of the four coronaviruses known to cause the common cold in humans, for example. It is not currently known, however, if the presence of IgG antibodies indicates immunity (or degree thereof) of the host against SARS-CoV-2. The duration of any conferred immunity, or the level of IgG antibodies required to effectively acquire such immunity, are also unknown. Additional research is needed and encouraged.

Lisboa Bastos et al. (2020) performed a meta-analysis to investigate the diagnostic accuracy of serological testing for COVID-19. The authors aimed to identify studies where serological testing was compared to the "reference standard of viral culture or reverse transcriptase polymerase chain reaction". The authors identified a total of 40 studies for inclusion in the study. The authors found the pooled sensitivity of enzyme linked immunosorbent assays (ELISAs) measuring IgG or IgM to be 84.3% (with a 95% confidence interval [CI] of 75.6%-90.9%). For lateral flow immunoassays (LFIAs), the pooled

sensitivity was found to be 66% (95% CI: 49.3%-79.3%), and for chemiluminescent immunoassays (CLIAs), the pooled sensitivity was found to be 97.8% (95% CI: 46.2%-100%). Pooled specificities ranged from 96.6%-99.7%. Sensitivity was also found to be higher at least three weeks from symptom onset (69.9% to 98.9%) compared to within the first week (13.4% to 50.3%) Of the samples used to calculate specificity, 83% were "from populations tested before the epidemic or not suspected of having COVID-19". The authors performed 49 bias risk assessments (one for methodology and one for patient selection) and identified 48 with a "high risk of patient selection bias" and 36 with "high or unclear risk of bias from performance or interpretation of the serological test". The authors also noted that only four of the forty studies including outpatients and only two studies evaluated point-of-care testing. The authors concluded that "currently, available evidence does not support the continued use of existing point-of-care serological tests" but acknowledged that "higher quality clinical studies assessing the diagnostic accuracy of serological tests for covid-19 are urgently needed" (Lisboa Bastos et al., 2020).

Kontou et al. (2020) performed a meta-analysis investigating the use of antibody tests in detecting SARS-CoV-2. The authors focused on IgG and IgM tests based on enzyme-linked immunosorbent assays (ELISA), chemiluminescence enzyme immunoassays (CLIA), fluorescence immunoassays (FIA), and lateral flow immunoassays (LFIA). A total of 38 studies encompassing 7848 individuals (3522 COVID-19 cases, 4326 healthy controls) were included. Of the 38 studies, 21 included data for both COVID-19 cases and controls. Fourteen studies using ELISA were included, and the authors found that IgG and IgM perform "similarly" individually, but in combination, resulted in a sensitivity of 0.935. Thirteen studies using CLIA resulted in an IgG sensitivity of 0.944, an IgM sensitivity of 0.810, and a combined IgG/IgM sensitivity of 0.910. The specificities ranged from 0.954 to 0.984. Thirteen studies used LFIA and found the IgG and IgM sensitivities to range from 0.53-0.66. Combining IgG and IgM resulted in sensitivities of 0.78-0.83. The authors also attempted to analyze FIA-based studies but were unable to due to the paucity of studies (three identified). The authors concluded that ELISA- and CLIA-based testing performed better sensitivity-wise and that LFIA studies are "more attractive for large seroprevalence studies but show lower sensitivity". (Kontou et al., 2020)

Ko et al. (2020) investigated the differences in neutralizing antibody production between asymptomatic and "mild" symptomatic COVID-19 patients, compared to pneumonic COVID-19 patients. A total of 70 patients (15 asymptomatic, 49 mild symptomatic, and 6 pneumonic) were included. A microneutralization assay was performed, along with a FIA and ELISA. Neutralizing antibody production was observed in all the pneumonic patients, 93.9% of the mildly symptomatic patients, and 80% of the asymptomatic patients. Further, the entire pneumonic group showed "high" titer (defined as ≥1:80), while 36.7% of the mild group and 20% of the asymptomatic group showed high titer. Both the FIA (for IgG) and ELISA detected anti SARS-CoV-2 at a high sensitivity (98.8% and 97.6% respectively). The authors concluded that "Most asymptomatic and mild COVID-19 patients produced the neutralizing antibody, although the titers were lower than pneumonia patients" (Ko et al., 2020).

Wu et al. (2020) investigated the association between levels of neutralizing antibodies (NAbs) and clinical characteristics in recovered COVID-19 patients. A total of 175 patients with "mild" symptoms of COVID-19 were included. The authors found that NAbs were detected in patients starting in days 4-6 and reached peak levels in days 10-15. NAbs were also found not to cross-react with SARS-associated CoV,but correlated with "spike-binding antibodies targeting S1, receptor binding domain, and S2 regions. The authors also noted that NAbs titers were "significantly" higher in 56 "older" patients (1537 [IQR, 877-2427]) and 63 "middle-aged" patients (1291 [IRQ, 504-2126]) compared to 56 "younger patients" (459 [IQR, 225-998]). The authors concluded that "…NAb titers to SARS-CoV-2 appeared to vary substantially. Further research is needed to understand the clinical implications of differing NAb titers for protection against future infection" (Wu et al., 2020).

Kweon et al. (2020) collected 97 samples from patients with COVID-19 to analyze the serologic profiles and time kinetics of IgG and IgM against SARS-CoV-2 using the AFIAS COVID-19 Ab (BodiTechMed, 2020) and the EDI™ Novel Coronavirus COVID-19 ELISA Kit (EpitopeDiagnostics, 2020). The AFIAS assay uses recombinant nucleocapsid protein as an antigen to determine IgG and IgM antibodies against

SARS-CoV-2 within 20 minutes from whole blood, serum, or plasma. The EDI™ ELISA Kit uses the microplate-based enzyme immunoassay technique to detect antibodies by measuring the optical densities (ODs) of each well of immunocomplexes. To determine the kinetics of antibodies, studies were performed at different past symptom onset (PSO) periods and to determine diagnostic accuracy of serologic assays, diagnostic sensitivity and specificities were calculated by PSO of ≤14 days and >14 days. Kinetic studies showed that "with both assays, IgM and IgG rapidly increased after 7 days post symptom onset (PSO). IgM antibody levels reached a peak at 15–35 d PSO and gradually decreased. IgG levels gradually increased and remained at similar levels after 22–35 d" (Kweon et al., 2020). The diagnostic accuracy of both serologic assays also differed based on PSO. "The sensitivity of IgG samples from ≤14 d PSO was as low as 35.7%~57.1%, but it sharply increased for >14 d PSO to 88.2%~94.1%. This means that almost all patients with COVID-19 showed seroconversion after 14 d PSO, and IgG seronegative subjects in this period are considered less likely to be infected with SARS-CoV-2. In addition, both assays showed 94.2~96.4% of IgG specificities and increased IgG titers in COVID-19 patients were maintained. Thus, IgG serologic assays can be useful for ruling out SARS-CoV-2 infection after 14 d PSO, detecting past infection, and epidemiologic surveys" (Kweon et al., 2020). For IgM, the sensitivities were "as low as 21.4% (same in both assays) in the samples collected ≤14 d PSO and 41.2%~52.9% in samples >14 d PSO. These findings indicated that in patients infected with SARS-CoV-2, IgM seroconversion may not develop or might not be detected until the middle or late stages of infection. In other words, SARS-CoV-2 infection may be missed based on IgM seropositivity; thus, IgM tests must not be solely used in COVID-19 diagnosis and should be used only as a supportive tool in addition to molecular tests" (Kweon et al., 2020). In addition, IgM titers in COVID-19 patients showed a significant reduction after 35 d PSO; therefore, their utility in detecting past infection is limited. The author concludes that "testing for antibodies against SARS-CoV-2, especially IgG, has the potential for ruling out SARS-CoV-2 infection after 14 d PSO, detecting past infection, and epidemiologic surveys" (Kweon et al., 2020).

Caturegli et al. (2020) performed a case-control study to determine the clinical utility and validity of using SARS-CoV-2 antibodies, which were serum IgG and IgA antibodies formed against the SARS-CoV-2 spike protein detected by enzyme-linked immunosorbent assay (ELISA). When assays were formed 14 days or later after symptom onset, the researchers found that the sensitivity was 0.976 (95% CI, 0.928 to 0.995) and specificity was 0.988 (95% CI, 0.974 to 0.995), but the sensitivity decreased at earlier time points. Antibodies "predicted the odds of developing acute respiratory distress syndrome, which increased by 62% (CI, 48% to 81%; P < 0.001) for every 2-fold increase in IgG." This demonstrates the linkage of antibodies used to measure clinical severity and for those who tested negative by NAAT but remained potentially COVID-positive.

In a household cohort study, Churiwal assessed the utility of a rapid point of care test for COVID-19 antibodies by comparing the performance of BioMedomics COVID-19 IgM/IgG Rapid Antibody Test against an ELISA. The test was performed on 303 patients at study enrollment and 4 weeks later. According to the results, sensitivity was lower early in infection and those who never developed symptoms (74% sensitivity). Only two were detected among 499 tests early in infection due to false-positive IgM bands. When measured 4 weeks later after the onset of symptoms, it demonstrated robust sensitivity (90%) and complete specificity (100%). The authors conclude that "When used appropriately, rapid antibody tests offer a convenient way to detect symptomatic infections during convalescence" (Churiwal et al., 2021).

Antigen Testing

Another possible diagnostic testing methodology is antigen detection testing, which relies upon the direct detection of parts of the virus called "antigens"—in this instance, proteins located on the outside of SARS-CoV-2, such as the spike protein (S) or nucleocapsid protein, that can cause an immune response in an individual. What makes this method of testing distinct from antibody testing is that antigen testing directly measures the presence of the virus in a person whereas antibody testing is measuring the patient's response to an infection. These antigen detection tests can be deployed as rapid antigen tests that decrease the turnaround time for results but usually lack specificity (Loeffelholz & Tang, 2020).

On May 8, 2020, the FDA issued the first EUA for antigen testing for COVID-19 to the Quidel Corporation for their Sofia®2 SARS Antigen FIA lateral flow immunofluorescent sandwich assay for the qualitative detection of the nucleocapsid (N) protein antigen of SARS-CoV-2 for use in individuals suspected of COVID-19 by their healthcare provider (Quidel_Corporation, 2020). This test has been approved as a point-of-care (POC) test (FDA, 2022c). This test functions by detecting the N protein of either the SARS-CoV or SARS-CoV-2 virus from an upper respiratory sample (either a nasal swab or nasopharyngeal swab). First, the sample is placed in a reagent tube so that any virus, if present, is broken apart to allow for the N proteins to be exposed. The sample then travels from the sample well, down a test strip—where the term "lateral flow" is derived—where the proprietary reagents will recognize any N proteins and trap them in place on the strip. The test requires at least 15 minutes to develop prior to analysis. The strip can then be read by the Sofia®2 system that measures the fluorescent signal from the proprietary reagents. The Sofia®2 system allows the user to have two different modes for analysis—"Walk Away" and "Read Now". For the "Walk Away" mode, the user will insert the test cassette strip into the system, and the results will be displayed in 15 minutes because the test will be developed while in the instrument. In "Read Now" mode, the user must have already allowed at least 15 minutes for the test to develop prior to inserting it into the instrument. Then, the Sofia®2 system will display the result within one minute (Quidel_Corporation, 2020). On August 20, 2020, Quidel reported that the Sofia test's labeling had been amended to include "either nasal or nasopharyngeal swabs" thereby allowing Quidel a second corresponding kit configuration (BioSpace, 2020).

On July 2, 2020, a second antigen test (BD Veritor System for Rapid Detection of SARS-CoV-2) from Becton, Dickinson, and Company was issued an EUA. This test is described as "a chromatographic digital immunoassay intended for the direct and qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasal swabs from individuals who are suspected of COVID-19 by their healthcare provider within the first five days of the onset of symptoms". The test is authorized for use in point-of-care (POC) settings. The test's mechanism of action is as follows: if there are any antigens in the sample (in this case, the nucleocapsid of the virus), they will bind to antibodies conjugated to detector particles in the test strip. The new "conjugates" migrate to the "reaction area" and are captured by another line of antibodies. The test reads positive when the conjugate is found at both "Control" and "Test" positions on the device. BD Veritor reported the following values for the test (in comparison to RT-PCR): 84% positive predictive agreement, 100% negative predictive agreement, 98% overall percent agreement, 100% positive predictive value, and 97.5% negative predictive value. No cross-reactivity was reported (BD_Veritor, 2020).

On August 18, 2020, a third antigen test (LumiraDx SARS-CoV-2 Ag Test from LumiraDx UK Ltd.) was issued an EUA. The test is described as "a single use fluorescence immunoassay device designed to detect the presence of the nucleocapsid protein antigen directly from SARS-CoV-2 in nasal swab specimens, without transport media". The mechanism of action is as follows: when a droplet of the specimen is added to the "Test Strip", pre-made reagents on the strip react with any antigen in the specimen. The amount of fluorescence created is proportional to the amount of antigen detected. LumiraDx reported a limit of detection of 32 $TCID_{50}$/mL [tissue-culture infectious dose], as well as a 97.6% positive percent agreement, 96.6% negative percent agreement, 93.1% positive predictive value, 98.8% negative predictive value, and 96.9% overall percent agreement (based on 257 total samples) (LumiraDx, 2020).

As of April 20, 2022, 50 antigen tests have Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA) (FDA, 2022d). These testing methods include (among others): Bulk Acoustic Wave (BAW) Biosensors, Chemiluminescence Immunoassays, Chromatographic Digital Immunoassays, Digital Lateral Flow, Magnetic Force-assisted Electrochemical Sandwich Immunoassay (MESIA), Microfluidic Immunofluorescence Assay, and Paramagnetic Microbead-based Immunoassay (FDA, 2022d).

Clinical Utility and Validity of Antigen Testing

To address the clinical performance, two primary studies were performed. Both studies only used frozen samples. The first study used 143 samples with 80% PPA or Positive Percent Agreement (47/59 of positive samples tested "positive"). They report 100% NPA or Negative Percent Agreement—all 84 negative samples tested "negative". The second study used a total of 48 samples. Again, 80% of the positive samples tested "positive"; however, only a total of five positive samples were included within this second study. The remaining 43 samples were all negative samples. This study reports a sensitivity of 80.0%, but a 95% confidence interval range of 37.6% - 96.4%. A third supportive study was also performed. In this study, thirty swabs were taken. Twenty of these swabs were spiked with one lower concentration of the virus while the remaining ten swabs were spiked with a higher concentration of the virus. Then, all 30 swabs were tested and compared to 47 control ("unspiked") samples. In this study, none of the "unspiked" control samples tested "positive" while all 30 of the "spiked" samples, regardless of the concentration, tested positive. Quidel also tested the limit of detection (LoD) of the Sofia®2 SARS Antigen FIA test. LoD is typically measured by determining the $TCID_{50}$ (median tissue culture infective dose). The $TCID_{50}$ is the amount where 50% of the cells within a sample are infected.(Wulff et al., 2012) For the Sofia®2 SARS Antigen FIA test, the LoD for a direct swab sample has a $TCID_{50}$ of 113 mL whereas it is 850 mL if the initial sample is from a swab sample that has been diluted into 3 mL of reagent. Finally, Quidel also checked this antigen test for possible cross-reactivity with a number of microorganisms and other viruses. It shows no cross-reactivity with any of the microorganisms or viruses tests other than SARS-CoV. Of note, it does not cross-react with human coronavirus 229e, OC43, NL63, or MERS-CoV (heat-inactivated); however, they did not check for possible cross-reactivity with the other known human coronavirus (HKU1) due to a lack of availability at this time. This is noteworthy since this coronavirus is associated with the common cold. Limitations of the Sofia®2 SARS Antigen FIA test includes the following:

- This test must be performed using the Sofia®2 system, and the test must be performed accurately following the test procedure. Failure to do so can adversely affect the performance of the test and may invalidate the results.
- A positive test cannot distinguish between a SARS-CoV or a SARS-CoV-2 infection. SARS-CoV is the virus that caused the SARS outbreak of 2003. It should be noted that there is no current outbreak of SARS.
- This test also does not distinguish between "live" (viable) virus and non-viable virus. Consequently, the test results do not necessarily correlate with viral culture results performed on the same sample.
- This test is only for the qualitative use on a sample from either a nasal swab or a nasopharyngeal swab. It has not been approved for use, at this time, on any other sample, such as saliva.
- Negative test results can occur if the viral level is below the lower limit of the test. All negative results "should be treated as presumptive and confirmed with an FDA authorized molecular assay, if necessary, for clinical management, including infection control"(Quidel_Corporation, 2020)
- Positive test results do not rule out co-infections, and negative results do not "rule in" other non-SARS viral or bacterial infections.
- The clinical performance assays submitted for FDA approval were performed using frozen samples; the test may have a different performance when used with a fresh sample (such as in a point-of-care setting).
- "If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments is required (Quidel_Corporation, 2020)."
- As previously noted, the company did not check this test (as of publication date) for cross-reactivity with human coronavirus HKU1 due to a lack of availability of that strain. This is notable since this particular virus is associated with upper respiratory conditions such as the common cold.

One multi-center study, currently a preprint at the time of publication, reports the development of another

rapid antigen detection test (RADT) that screens for SARS-CoV-2 by targeting the nucleocapsid protein. This test, when using a nasopharyngeal swab sample, reports a 100% positive agreement with RT-PCR testing. They also report 73.6% positive agreement when using a urine sample (Diao et al., 2020). This study is yet to be published in a peer-reviewed journal, and the test is not FDA-approved as of May 18, 2020. Another study published recently in *ACS Nano* reports on the development of a RADT using field-effect transistor (FET)-based biosensing where a graphene sheet for the FET is coated with a specific antibody against the SARS-CoV-2 spike protein. This method can detect the protein in concentrations as low as 1 fg/mL in buffer and has an LOD of 242 copies/mL for a clinical sample (versus 16/mL for a culture medium) (Seo et al., 2020). To date, the WHO states that "Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19. Currently, there is insufficient evidence on performance and operational use to recommend specific commercial products" (WHO, 2021a).

Scohy et al. (2020) evaluated the Coris COVID-19 Ag [Antigen] Respi-Strip test in comparison to RT-PCR. The authors tested 148 nasopharyngeal swabs, with 106 testing positive by RT-PCR. The rapid antigen test detected 32 of these 106 positive results, for a sensitivity of 30.2%. All samples deemed positive by the antigen test were also deemed positive by RT-PCR. The authors noted that higher viral loads were associated with better detection by antigen tests but concluded that "the overall poor sensitivity of the COVID-19 Ag Respi-Strip does not allow using it alone as the frontline testing for COVID-19 diagnosis" (Scohy et al., 2020).

Mak et al. (2020) evaluated the BIOCREDIT COVID-19 Ag test in comparison to RT-PCR. The BIOCREDIT test's limit of detection (LOD) was compared to RT-PCR and viral culture, and a total of 368 samples from confirmed COVID-19 cases were included. A sample volume of 100 μL was used. The authors found the LOD of BIOCREDIT to be 1000-fold less sensitive than viral culture (BIOCREDIT LOD: 10^{-2}, viral culture: 10^{-5}). RT-PCR's LOD was measured to be 10^{-7}. Further, BIOCREDIT detected between 11.1% and 45.7% of RT-PCR positive patients from COVID-19 patients. The authors concluded that "This study demonstrated that the RAD test serves only as adjunct to RT-PCR test because of potential for false-negative results" (Mak et al., 2020).

Lambert-Niclot et al. (2020) analyzed the COVID-19 Ag Respi-Strip test and compared its accuracy to RT-PCR. A total of 138 nasopharyngeal samples were included, with 94 testing positive by RT-PCR. The Respi-Strip test identified 47 of 94 positive specimens for a sensitivity of 50%, although the specificity was 100% for both tests. The authors also noted that the control lines were "barely" visible for 17 tests (9 positive and 8 negative). The authors acknowledged that due to the low prevalence in France (the country in which this study was performed), prospective studies should be undertaken(Lambert-Niclot et al., 2020).

Hirotsu et al. (2020) evaluated a new antigen test (LUMIPULSE) which is based on chemiluminescence enzyme immunoassay. A total of 313 nasopharyngeal swabs were included (82 serial samples from 7 COVID patients, 231 individual samples from 4 COVID patients and 215 healthy controls). These samples were tested by both LUMIPULSE and RT-PCR. Compared to RT-PCR, LUMIPULSE demonstrated a 91.4% overall agreement rate (286/313), with a 55.2% sensitivity and 99.6% specificity. At >100 viral copies, LUMIPULSE agreed perfectly with RT-PCR, and at 10-100 viral copies, there was an 85% concordance rate (with concordance declining at lower viral loads). The authors concluded that "the LUMIPULSE antigen test can rapidly identify SARS-CoV-2-infected individuals with moderate to high viral loads and may be helpful for monitoring viral clearance in hospitalized patients" (Hirotsu et al., 2020).

Villaverde et al. (2021) conducted a multicenter study to compare the diagnostic accuracy of the Panbio coronavirus disease 2019 Antigen Rapid Test of nasopharyngeal samples in pediatric patients with COVID-19 symptoms ≤5 days. They demonstrated "limited accuracy in nasopharyngeal antigen testing: overall sensitivity was 45.4%, and 99.8% of specificity, positive-predictive value was 92.5%," with moderate concordance between the RT-PCR and antigen test. They noted that a high proportion of false-

negative results from the antigen tests (54.5%) may have public health implications in unknown spreading of the virus. But because this test has a good positive likelihood ratio, and is cheap, rapid, and widely distributed, it may be used as a first screening test in a pandemic situation, though its value as a diagnostic tool is questionable due to the low sensitivity and negative likelihood ratio.

Peacock studied the clinical utility of the BinaxNOW antigen test by Abbott Diagnostics, a lateral flow immunochromatographic point-of-care test which provides results in 15 minutes from a nasal swab. BinaxNOW was performed on 735 samples and results were compared to PCR. 623 of 735 (84.8%) had symptoms and 460 of 623 patients (62.6%) had symptoms for less than 7 days. Positive tests occurred in 173 (23.5%) for the PCR and 141 (19.2%) with the BinaxNOW test. Those with symptoms for more than 2 weeks had a positive test rate half of those with earlier onset. " In patients with symptoms ≤7 days, the sensitivity, specificity, and negative and positive predictive values for the BinaxNOW test were 84.6%, 98.5%, 94.9%, and 95.2%, respectively" (Peacock et al., 2022). The authors conclude that BinaxNOW has good sensitivity and specificity and is recommended for patients with symptoms up to 2 weeks (Peacock et al., 2022).

Panel Testing

Multiple laboratories have developed panels to screen for possible microorganism infections from a single sample. For example, multiplex polymerase chain reaction (PCR) can simultaneously detect multiple pathogens rather than sequentially testing for each individual pathogen. Such testing can be advantageous when different pathogens may manifest with similar clinical presentation; however, this testing can be costly and can also result in false-negatives if preferential amplification of one target over another occurs (Palavecino, 2015). As of May 4, 2022, the BioFire® Respiratory Panel 2.1 (RP2.1), the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, ePlex Respiratory Pathogen Panel 2, cobas SARS-CoV-2 & Influenza A/B, Xpert Xpress SARS-CoV-2/Flu/RSV, Quest Diagnostics RC COVID-19 +Flu RT-PCR, Sofia 2 Flu + SARS Antigen FIA, and the Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay from the CDC received an EUA from the FDA for testing for COVID-19 (FDA, 2022c). The BioFire® Respiratory Panel 2.1, the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, and ePlex Respiratory Pathogen Panel 2 use multiplex nucleic acid testing from a nasopharyngeal swab to detect and differentiate microorganisms listed in **Table 1** (BioFire, 2020; GenMark_Diagnostics, 2020; Qiagen_GmbH, 2020), whereas the CDC Multiplex detects and differentiates influenzas A and B from SARS-CoV-2 (CDC, 2020c).

Table 1: Respiratory Pathogen Panel Testing Containing SARS-CoV-2

BioFire® Respiratory Panel 2.1	QIAstat-Dx® Respiratory SARS-CoV-2 Panel	ePlex Respiratory Pathogen Panel 2

• **Adenovirus**
• **HCoV 229E**
• **HCoV HKU1**
• **HCoVNL63**
• **HCoV OC43**
• **SARS-CoV-2**
• **Human Metapneumovirus**
• **Human Rhinovirus/Enterovirus**
• **Influenza A**
  ○ **Subtype H1**
  ○ **Subtype H3**
  ○ **Subtype H1-2009**
• **Influenza B**
• **Parainfluenza Virus 1**
• **Parainfluenza Virus 2**
• **Parainfluenza Virus 3**
• **Parainfluenza Virus 4**
• **Respiratory Syncytial Virus**
• ***Bordetella parapertussis***
• ***Bordetella pertussis***
• ***Chlamydia pneumoniae***
• ***Mycoplasma pneumoniae***
	• Adenovirus
	• HCoV 229E
	• HCoV HKU1
	• HCoVNL63
	• HCoV OC43
	• SARS-CoV-2
	• Human Metapneumovirus A+B
	• Influenza A
	  ○ Subtype H1
	  ○ Subtype H3
	  ○ Subtype H1N1/pdm09
	• Influenza B
	• Parainfluenza Virus 1
	• Parainfluenza Virus 2
	• Parainfluenza Virus 3
	• Parainfluenza Virus 4
	• Rhinovirus/Enterovirus
	• Respiratory Syncytial Virus A+B
	• *Bordetella pertussis*
	• *Chlamydia pneumoniae*
	• *Mycoplasma pneumoniae*
		• Adenovirus
		• HCoV 229E
		• HCoV HKU1
		• HCoVNL63
		• HCoV OC43
		• SARS-CoV-2
		• Human Metapneumovirus A+B
		• Influenza A
		  ○ Subtype H1
		  ○ Subtype H3
		  ○ Subtype H1-2009
		• Influenza B
		• Parainfluenza Virus 1
		• Parainfluenza Virus 2
		• Parainfluenza Virus 3
		• Parainfluenza Virus 4
		• Rhinovirus/Enterovirus
		• Respiratory Syncytial Virus A+B
		• *Chlamydia pneumoniae*
		• *Mycoplasma pneumoniae*

Clinical Utility and Validity of Panel Testing

The BioFire RP2.1 panel must be used with either the BioFire FilmArray 2.0 or BioFire FilmArray Torch Systems, and it does not provide a quantitative value for any particular organism within the sample. This panel "has not been established for specimens collected from individuals without signs or symptoms of respiratory infection (BioFire, 2020)." This panel has not been validated for the monitoring of treatment for any condition. If a test result shows four or more organisms detected, then the sample should be retested. A negative result does not necessarily exclude an infection. "Negative test results may occur from the presence of sequence variants (or mutation) in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, an infection caused by an organism not detected by the panel, or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen (BioFire, 2020)."

The BioFire RP2.1 panel cannot necessarily distinguish between existing viral strains and new variants. One example is the inability to distinguish between Influenza A H3N2v and seasonal Influenza A H3N2. This panel also cannot reliably differentiate between human rhinovirus and enterovirus due to genetic similarity. If detected, the "result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required (BioFire, 2020)." The performance characteristics of several microorganisms detected by this panel, including HCoV 229E, were determined using retrospective clinical specimens due to the small number of positive specimens collected. The BioFire RP2.1 panel should not be used if *B. pertussis* is suspected because of its low sensitivity. "[A]

B. pertussis molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to *B. pertussis* only should be used instead (BioFire, 2020)." This is because the RP2.1 panel targets a single-copy promoter target (*ptxP*) whereas more sensitive tests target the multi-copy IS*481* insertion sequence. The BioFire RP2.1 panel also shows cross-reactivity with *B. bronchiseptica* and *B. parapertussis* at higher concentrations.

The primers used in the BioFire RP2.1 panel to detect COVID-19 may cross-react with coronaviruses from other species due to high sequence homology. BioFire reports predicted cross-reactivity with up to three bat coronaviruses (accession: MN996532, MG772933, and MG772934) and one pangolin coronavirus (accession: MT084071). However, "[i]t is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (BioFire, 2020)."

The difference between the BioFire RP2 panel and the BioFire RP2.1 panel is the ability to detect SARS-CoV-2. Consequently, within the Instructions for Use (IFU) for the RP2.1 panel, BioFire reports on the data of the RP2 panel. The clinical performance of the RP2 panel was determined using both fresh and frozen samples. The clinical performance values for the four endemic HCoVs are listed in **Table 2** (BioFire, 2020). They note a cross-reactivity between HCoV-OC43 and HCoV-HKU1.

Table 2: Clinical Performance of BioFire RP2/RP2.1 Panel for Endemic HCoVs

Analyte	PPA	PPA 95% CI	NPA	NPA 95%CI
HCoV-229E	11/12 (91.7%)	64.6 – 98.5	1595/1600 (99.7%)	99.3 – 99.9
HCoV-HKU1	43/43 (100%)	91.8 – 100	1557/1569 (99.2%)	98.7 – 99.6
HCoV-NL63	40/40 (100%)	91.2 – 100	1562/1572 (99.4%)	98.8 – 99.7
HCoV-OC43	33/41 (80.5%)	66.0 – 89.8	1566/1571 (99.7%)	99.3 – 99.9
Notes: Abbreviations used—PPA (Positive Percent Agreement); NPA (Negative Percent Agreement).				

Concerning the detection of SARS-CoV-2, the BioFire RP2.1 panel reports a limit of detection (LoD), using the USA-WA1/2020 isolate, of 500 copies/mL when using a heat-inactivated virus. They report a 100% detection rate (20/20). This equates to 6.9 X 10^{-2} $TCID_{50}$/mL. They also tested the LoD using an infectious virus isolate obtained from the World Reference Center for Emerging Viruses and Arboviruses, contributed by the CDC. With this infectious sample, the LoD was determined to be 160 copies/mL (or 1.1 X 10^{-2} $TCID_{50}$/mL). Again, they report a 100% detection rate (20/20) (BioFire, 2020).

Similar to the BioFire panel test, the QIAstat-Dx Respiratory SARS-CoV-2 panel test by Qiagen is for use on a proprietary system, the QIAstat Dx Analyzer System. It is also a qualitative test approved for testing in "patients suspected of COVID-19 by their healthcare provider". It is also "not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions" (Qiagen_GmbH, 2020). It is important to note that the test performance in either immunocompromised individuals or asymptomatic individuals has not been established as of publication date. A positive test result cannot rule out a co-infection; an erroneous negative test result can be due to erroneous sample handling as well as variations in the target sequences, organism levels below the limits of detection, and/or use of an interfering reagent (such as certain medications or therapies). Since the QIAstat-Dx test targets the *E* gene of SARS-CoV-2, which is homologous to sequences in multiple bat SARS viruses, it is possible to cross-react with these bat SARS viruses; however, the likelihood of infection of these viruses in humans

is unlikely since none have been reported to date (Qiagen_GmbH, 2020).

Also, like the BioFire RP2/RP2.1 panel tests, the QIAstat-Dx test may not distinguish between existing viral strains and emerging viral strains, such as influenza A. However, unlike the BioFire RP2/RP2.1 panel tests, the QIAstat-Dx test does detect the IS*481* multi-copy insertion sequence present in multiple *Bordetella* species. This does increase the sensitivity of the test, but it can increase the possibility of false-positive results if the specimen is contaminated with a non-pertussis *Bordetella* species (Qiagen_GmbH, 2020).

In addressing the clinical performance of the QIAstat-Dx test for detecting SARS-CoV-2, Qiagen set up two positive trials (one at a higher concentration sample [n = 10] and one at a low positive contrived sample [n = 20), and they report a positive percent agreement (PPA) of 100% (30/30) (95% CI: 85.8 – 100%). Likewise, they did a negative control (n = 30) and report a negative percent agreement (NPA) of 100% (30/30) (95% CI: 85.8 – 100%). In reporting the limit of detection (LoD), they used 20 replicates with a detection rate of at least 95% (or 19/20) to generate a 'positive' signal. Using source material obtained from the clinical sample strain of the Hospital of Barcelona (Spain), Qiagen reports an LoD of 500 copies/mL.

The performance of the other targets within the panel were assessed in a multi-center study conducted at six geographically diverse study sites—Copenhagen, Denmark; Minneapolis, MN; Indianapolis, IN; Liverpool, NY; Columbus, OH; and Albuquerque, NM. The performance was determined using both frozen and fresh samples. The clinical performance values for the four endemic HCoVs are listed in **Table 3 (Qiagen_GmbH, 2020)**.

Table 3: Clinical Performance of QIAstat-Dx Panel for Endemic HCoVs

Analyte	PPA	PPA 95% CI	NPA	NPA 95%CI
HCoV-229E	8/9 (88.9%)	56.5 – 98.0	1975/1975 (100%)	99.8 – 100.0
HCoV-HKU1	51/52 (98.1%)	89.9 – 99.7	1925/1932 (99.6%)	99.3 – 99.8
HCoV-NL63	40/47 (85.1%)	72.3 – 92.6	1936/1938 (99.9%)	99.6 – 100.0
HCoV-OC43	26/29 (89.7%)	73.6 – 96.4	1951/1955 (99.8%)	99.5 – 99.9
Notes: Abbreviations used—PPA (Positive Percent Agreement); NPA (Negative Percent Agreement).				

As with the other two tests, the ePlex RP2 Panel "should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of active infection with the identified respiratory pathogen but do not rule out infection or co-infection with non-panel organisms. The agent detected by the ePlex RP2 Panel may not be the definite cause of disease. Negative results for SARS-CoV-2 and other organisms on the ePlex RP2 Panel may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Negative results do not preclude infection with SARSCoV-2 or other organisms on the ePlex RP2 Panel and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information (GenMark_Diagnostics, 2020)." A limitation of ePlex RP2 Panel is its unpredictability in differentiating human rhinovirus and enterovirus due to genetic similarity. If differentiation is required, an ePlex RP2 Panel positive human rhinovirus/enterovirus result should be followed-up using an alternative method, such as cell culture or sequence analysis. Cross-reactivity with SARS-CoV-1 is also observed at high titers.

To test the performance characteristics of ePlex RP2 Panel for SARS-CoV-2 detection, 170 nasopharyngeal previously frozen swab samples were collected (59 known SARS-CoV-2 positive and 111 presumed SARSCoV-2 negative samples). "Positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of TP and false negative (FN) results, while negative percent agreement (NPA) was calculated by dividing the number of true negative (TN) results by the sum of TN and false positive (FP) results" (GenMark_Diagnostics, 2020). The ePlex RP2 Panel detected SARS-CoV-2 in 59/59 positive specimens (100% positive percent agreement) and confirmed 111/111 negative specimens (100% negative percent agreement). To determine the limit of detection (LoD), the lowest concentration at which SARS-CoV-2 is detected at least 95% of the time, serial dilutions were prepared in a natural clinical matrix and at least 20 replicates per concentration were tested in the study. "The LoD concentration for detection of SARS-CoV-2 was determined to be 0.01 $TCID_{50}$/mL, which corresponds to 250 genomic copies per milliliter, as determined by digital droplet PCR (GenMark_Diagnostics, 2020)."

Regarding the "Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay" from the CDC, the FDA reported a limit of detection (LOD) of 1.01×10^{-2} (at ID_{50} [infective dose] / reaction). The panel was evaluated using 104 samples (33 positive for SARS-CoV-2, 30 positives for influenza A, and 30 positive for influenza B, 11 negative samples), and compared to an RT-PCR assay. There was a 100% concordance rate between the two tests. Additionally, cross-reactivity between the three analytes and 35 common respiratory pathogens (16 viruses, 18 bacterial species, 1 yeast) was evaluated, and no cross-reactivity was identified (FDA, 2020b).

The cobas SARS-CoV-2 & Influenza A/B panel is approved for emergency use authorization by the FDA; the panel uses qualitative detection of nucleic acids from SARS-CoV-2 in pooled samples. Six cultured viruses are tested for, two each of influenza A and influenza B strains as well as SARS-CoV-2. In an independent study, Poljak et al. (2020) performed a clinical evaluation of the cobas SARS-Cov-2 test (non-inclusive of influenza A/B panel). The cobas SARS-CoV-2 test was evaluated against an in-house and well-characterized comparator using 217 samples. cobas and the comparator showed overall agreement of 98.1%. Another comparative evaluation on 502 samples showed agreement of 99.6%. The authors concluded that cobas "is a reliable assay for qualitative detection of SARS-CoV-2 in nasopharyngeal swab samples collected in the Universal Transport Medium System (UTM-RT)(Poljak et al., 2020)."

There are other panels that are not yet FDA approved such as the AMPLIQUICK® Respiratory Triplex assay that detects and differentiates between SARS-CoV-2, influenza A/B and respiratory syncytial viruses in respiratory specimens. Results from AMPLIQUICK® were compared to the Allplex™ Respiratory Panel 1 and 2019-nCoV assays. 359 predetermined respiratory samples with diagnosed SARS-CoV-2, influenza A, influenza B and RSV were included in the study. The AMPLIQUICK® Respiratory Triplex "showed high concordance with the reference assays, with an overall agreement for SARS-CoV-2, influenza A, influenza B, and RSV at 97.6%, 98.8%, 98.3% and 100.0%, respectively." The authors conclude that the "AMPLIQUICK® Respiratory Triplex is a reliable assay for the qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and RSV in respiratory specimens, which may prove useful for streamlining diagnostics during the winter influenza-seasons" (Mboumba Bouassa et al., 2022).

Miscellaneous Testing

Other methodologies have been proposed to complement or even replace the standard tests described above. For example, a new "RT-LAMP" (reverse transcription loop-mediated isothermal amplification) application has started to see some use for the COVID-19 pandemic. This technique attempts to combine the speed of antigen testing and the accuracy of nucleic acid testing; RT-LAMP includes the traditional reverse transcriptase (RT), as well as a DNA polymerase with "strong strand displacement activity and tolerance for elevated temperatures and up to six DNA oligonucleotides of a certain architecture". These oligonucleotides act as primers for the RT, but additional oligonucleotides for the DNA polymerase are

designed so that the DNA products loop back into their ends. This results in "self-priming templates" for the DNA polymerase, which allows the reaction [the nucleic acid amplification] to proceed as normal. Detection of the amplified DNA without specialized instrumentation is the key challenge; some tests use a pH indicator that changes the color of the solution the reaction is run in. Since the reaction does not require the use of a thermal cycler with real-time fluorescence measurement, the results can be delivered in a faster time frame than traditional RT-PCRs (Dao Thi et al., 2020).

Nagura-Ikeda et al. (2020) evaluated the "clinical performance of six molecular diagnostic tests and a rapid antigen test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)". Self-collected saliva was the medium used for analysis. A total of 103 patients with COVID-19 were included (15 asymptomatic, 88 symptomatic). The six molecular diagnostic tests included three RT-PCR tests, an RT-qPCR test, a "cobas SARS-CoV-2 high-throughput system" and an RT-LAMP assay. The molecular diagnostic tests detected viral RNA in 50.5%-81.6% of specimens and an antigen was detected in 11.7% of the specimens by the rapid antigen test. Viral RNA was also detected at a higher rate (65.6%-93.4%) in specimens collected within 9 days of symptom onset compared to specimens collected after 10 days (22.2%-66.7%). Viral RNA was detected in asymptomatic patients at a rate of 40%-66.7%. The authors concluded "Self-collected saliva is an alternative specimen option for diagnosing COVID-19. LDT RT-qPCR…and RT-LAMP showed sufficient sensitivity in clinical use to be selectively used according to clinical settings and facilities. The rapid antigen test alone is not recommended for initial COVID-19 diagnosis because of its low sensitivity" (Nagura-Ikeda et al., 2020).

Dao Thi et al. (2020) performed a validation of a "two-color RT-LAMP assay protocol for detecting SARS-CoV-2 viral RNA using a primer set specific for the N gene". The authors wrote that a positive sample would be detected by a color change from red to yellow and tested their RT-LAMP assay on "surplus RNA samples isolated from 768 pharyngeal swab specimens collected from individuals being tested for COVID-19". The results were compared to a traditional RT-qPCR assay. The specificity of the RT-LAMP assay was found to be 99.7%. Further, the RT-qPCR positive samples with a cycle threshold (CT) number of under 30 scored positive (agreeance) in the RT-LAMP assay at a 97.5% agreeance rate. Agreeance rate declined both at the 30-35 threshold and at the 35-40 threshold. The authors also developed a "swab-to-RT" LAMP protocol, which was measured at 86% sensitivity (for CT <30) and a 99.5% specificity. The authors concluded that "The RT-LAMP assay and LAMP-sequencing extend the range of available test methods and complement individual tests and pooled tests based on RT-qPCR with a faster, simpler, and potentially more cost-effective test method" (Dao Thi et al., 2020).

R. Wang et al. (2020) demonstrated a one-pot visual SARS-CoV-2 detection system named "opvCRISPR" by integrating reverse transcription loop-mediated isothermal amplification (RT-LAMP) and Cas12a cleavage in a single reaction system, which simplifies operations and avoids contamination. The opvCRISPR enables detection at every single molecular level in forty-five minutes. "The RT-LAMP reagents are incubated at the bottom of the tube, and CRISPR/Cas12a reaction reagents are added on the lid. SARS-CoV-2 RNA templates extracted from the respiratory swab are amplified by RT- LAMP, followed by mixing with the Cas12a reagents for cleavage. Once the Cas12a nuclease is activated by recognizing DNA target, it splits the quenched fluorescent single-stranded DNA (ssDNA) reporter (FAM-TTATT-BHQ1) indiscriminately, generating the fluorescence signal visible to the naked eye under blue light" (R. Wang et al., 2020). To investigate the diagnostic accuracy of opvCRISPR, 26 SARS-CoV-2 RT-PCR positive respiratory swab samples and 24 SARS-CoV-2 RT-PCR negative samples were tested. "All infected samples were determined to be SARS-CoV-2 positive while all uninfected samples tested to be negative by both opvCRISPR and RT- PCR. The opvCRISPR diagnostic results provide 100% agreement with the Centers for Disease Control and Prevention (CDC)-approved quantitative RT-PCR assay" (R. Wang et al., 2020). The author states that "the proposed method only requires minimal equipment, demonstrating great potential in enabling next-generation molecular diagnosis towards point-of- care diagnosis. However, the present method requires additional step to extract RNA. Further efforts need to be made to combine the RNA extraction module with the opvCRISPR to achieve from sampling to result nucleic acid detection" (R. Wang et al., 2020).

Another methodology with potential application for COVID-19 testing is next-generation sequencing (NGS). The NGS procedure typically includes the following steps: first the patient's DNA is prepared to serve as a template, then DNA fragments are isolated (on solid surfaces such as small beads) where sequence data is generated, then these results are compared against a reference genome. Any DNA sample may be used if the quality and quantity of that sample are sufficient, but the methods of library generation and data analysis often vary from panel to panel. NGS is often used to produce swift and high-volume sequencing (Hulick, 2022). The FDA issued an EUA to Illumina, Inc. for the Illumina COVIDSeq Test on June 10, 2020 but has since updated its indications on October 29, 2020 to be for the "qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider" (FDA, 2021b). The FDA also issued an EUA to Helix OpCo LLC (dba Helix) for the Helix COVID-19 NGS Test on August 6, 2020. The test detects the gene for the SARS-CoV-2 spike protein, as well as one internal control (the human gene *RPP30*). The limit of detection was found to be 125 genetic copy equivalents / mL, and both the positive and negative percent agreements were measured to be 100% over 30 samples (Helix, 2020).

Furthermore, whole genome sequencing (WGS) has been demonstrated to have application for COVID-19 testing as well. WGS is conducted through four steps of DNA shearing, by using "molecular scissors" to cut DNA; then DNA bar-coding, for which "scientists add small pieces of DNA tags/bar codes to identify which piece of sheared DNA belongs to which [pathogen];" then the bar-coded DNA is put into the whole genome sequencer that identifies the bases; and finally, the data is analyzed to compare sequences and identify possible differences (CDC, 2016). In several countries, like the Netherlands, China, Vietnam, and the United States, particularly rapid WGS has been beneficial in informing outbreak response, general public health decision making, and infection risk in various facilities (Chau et al., 2021; Oude Munnink et al., 2020; Taylor et al., 2020; F. Wang et al., 2020). In the Netherlands, WGS with the first cases in February 2020 was able to confirm separate introductions of the virus into the country, and attribute increases in case prevalence to co-circulating virus variants following the spring holidays. WGS informed the sequence diversity that existed in Italy, which was where most COVID-positive individuals were returning from. The researchers concluded that "WGS in combination with epidemiological data strengthened the evidence base for public health decision-making in the Netherlands as it enabled a more precise understanding of the transmission patterns in various initial phases of the outbreaks. As such, we were able to understand the genetic diversity of the multiple introduction events in phase 1, the extent of local and regional clusters in phase 2 and the transmission patterns within the HCW [healthcare worker] groups in phase 3 (among which the absence or occurrence of very limited nosocomial transmission)" (Oude Munnink et al., 2020). In Vietnam, a similar application was made regarding a previously known strain responsible for a virus outbreak in the northern region. By whole genome sequencing, researchers were able to identify the first case of the B.1.1.7 variant from locally acquired infection. As the outbreak expanded, whole genome sequencing enabled enhanced surveillance in high risk groups, like those working in airports, who ended up being assigned another variant of A.23.1, as well as contact tracing and testing to detect more cases (Chau et al., 2021). In China, whole genome sequencing in this initial genomic study was able to provide insight towards the genotype-phenotype differences between COVID-19 positive patients. The researchers concluded, "Pedigree analysis suggested a potential monogenic effect of loss of function variants in GOLGA3 and DPP7 for critically ill and asymptomatic disease demonstration. Genome-wide association study suggests the most significant gene locus associated with severity were located in TMEM189–UBE2V1 that involved in the IL-1 signaling pathway…We identified that the HLA-A*11:01, B*51:01, and C*14:02 alleles significantly predispose the worst outcome of the patients" (F. Wang et al., 2020).

In the United States, a *Morbidity and Mortality Weekly Report (MMWR)* released in September 2020 utilized serial testing and virus whole genome sequencing at two skilled nursing facilities with COVID-19 outbreaks from April to June 2020 in Minnesota. From a total of 25 specimens from residents at the two different facilities, "strains from 17 residents and five HCP [health care personnel] were genetically similar, including one collected from a dietary worker with limited resident contact. Specimens from two

HCP and one resident at facility A had distinctly different virus sequences from the first cluster and from each other. At facility B, 75 (66%) resident specimens and five (7%) HCP specimens were sequenced, all of which were genetically similar," which suggested "intrafacility transmission." However, the limited participation by HCPs in serial testing could have "have biased identification of infections and limited interpretation of genomic sequencing" and limited "the description of genetic diversity" (Taylor et al., 2020). Generally, whole-genome sequencing still seems to have some limitations, in that "it still presents practical difficulties such as high cost, shortage of available reagents in the global market, need of a specialized laboratorial infrastructure and well-trained staff" resulting in "SARS-CoV-2 surveillance blackouts across several countries" (Bezerra et al., 2021). As of May 4, 2022, there are no FDA approved tests specifically for WGS.

Other types of specimens or media have been proposed as viable for COVID-19 testing, such as saliva. Saliva's primary advantages include its flexibility, its safety, and overall ease of use in testing. Santosh et al. also noted that To et al. found that saliva has a "high consistency rate of greater than 90% with nasopharyngeal specimens in the detection of respiratory viruses, including coronaviruses" (Sri Santosh et al., 2020; To et al., 2019). On August 15, 2020, the FDA issued an EUA to Yale School of Public Health for "SalivaDirect" which uses saliva samples for COVID-19 testing. Although this test still uses RT-PCR, the test still detects the nucleic acids in saliva, but does not require otherwise specialized or proprietary equipment for extraction of those nucleic acids. In the "Performance Evaluation" section of the official EUA, the FDA noted a positive agreement level between SalivaDirect and the ThermoFisher Scientific TaqPath COVID-19 combo kit to be 94.1% (32/34) and a negative agreement level to be 90.9% (30/33). (FDA, 2020a)

A third innovation in COVID-19 testing was published by the FDA on July 18, 2020. On this date, the FDA stated that they reissued an EUA to Quest Diagnostics to authorize Quest SARS-CoV-2 rRT-PCR test for use with "pooled" samples. This testing practice refers to testing multiple samples simultaneously, thereby allowing more efficient testing. The Quest SARS-CoV-2 rRT-PCR test was authorized to test up to 4 samples at once. The FDA notes that this strategy is most efficient in areas with low prevalence of COVID (i.e., most tests are expected to be negative). In the EUA, the FDA writes that if the "positivity rate" for any given individual to be tested is over 25%, the pooling strategy should not be used due to inefficiency (FDA, 2020c). Yelin et al. found that a single positive sample could be identified in pools of up to 32 samples (with a false negative rate of 10%) and noted that detection of a single positive sample in a pool of 64 samples may be possible with additional amplification cycles. (Yelin et al., 2020). Additional EUAs have been issued specifically for tests using pooled samples, such as the UCSD RC SARS-CoV-2 Assay (University of California San Diego Health, RT-PCR, 5 samples), the Poplar SARS-CoV-2 TMA Pooling assay (Poplar Healthcare, TMA [transcription-mediated amplification], 7 samples), and the "COVID-19 RT-PCR Test" (LabCorp, RT-PCR, 5 samples) (LabCorp, 2020a; Poplar, 2020; UCSD, 2020).

Hogan et al. (2020) performed an analysis of pooled sample analysis in a community setting. The authors analyzed samples in pools of 9 or 10, and the RT-PCR assay targeted the envelope (E) gene. When a positive pool was identified, each sample was tested individually for both the E gene and the RNA-dependent RNA polymerase (RdRp) gene for confirmation. The authors investigated 292 pools encompassing 2740 nasopharyngeal samples and 148 bronchoalveolar lavage samples. Two positive samples were identified (0.07%), which both showed detection of both genes. The authors identified one pool with a "positive E signal" that was not reproducible with testing individual samples of that pool. The authors did acknowledge that this methodology may miss individuals in which a COVID-19 risk has not been identified, but concluded that "strategies such as pooled screening may facilitate detection of early community transmission of SARS-CoV-2 and enable timely implementation of appropriate infection control measures to reduce spread (Hogan et al., 2020).

Another innovative technique in COVID-19 testing was announced on April 14, 2022, in an FDA press announcement. The InspectIR COVID-19 Breathalyzer is the first FDA Emergency Use Authorization-approved diagnostic test to use breath samples; the test detects chemical compounds in breath and

provides results in less than three minutes. According to the FDA, a validation study of 2,409 individuals (both symptomatic and asymptomatic) showed 91.2% sensitivity and 99.3% specificity for detecting COVID-19. In addition, the study evidenced a negative predictive value of 99.6% in a population composed of only 4.2% of people who were positive for the virus. A follow-up study with the Omicron variant showed similar sensitivity values (FDA, 2022b).

Guidelines and Recommendations

World Health Organization

The World Health Organization (WHO) published an interim guideline for the diagnostic testing of "2019 novel coronavirus [termed 2019-nCoV]" on September 11, 2020 (WHO, 2020a). First, they state that routine confirmation of COVID-19 cases is based on nucleic acid testing. Regarding serum testing, they remark that "if negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2-4 weeks later can be used to look for seroconversion or a rise in antibody titres." Finally, they recommend against viral culture or isolation as a routine diagnostic procedure and WHO does not recommend the use of saliva as the sole specimen type for routine clinical diagnostics (WHO, 2020a).

The WHO released a scientific brief with recommendations for the use of SARS-CoV-2 Ag-RDTs and updated their interim guidance on October 6, 2021. Within the guidelines, "SARS-CoV-2 Ag-RDTs (antigen detecting rapid diagnostic tests) that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay can be used to diagnose SARS-CoV-2 in suspected COVID-19 cases" (WHO, 2021a). Ag-RDTs should be conducted within 5-7 days after the onset of symptoms, as "patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher." WHO recommends that Ag-RDTs be used in settings when they are most reliable – in areas "when there is ongoing community transmission (≥5% test positivity rate). When there is no transmission or low transmission, the positive predictive value of Ag-RDTs will be low (many false positives), and in this setting NAAT is preferable as the first-line testing method or for confirmation of positive Ag-RDTs."

WHO does not recommend using SARS-CoV-2 Ag-RDTs when:

- "Symptomatic individuals (suspected COVID-19 cases) in the first 5-7 days since onset of symptoms"
- For asymptomatic individuals, only "limited to contacts of confirmed or probable cases and to at-risk health workers until more evidence is available on the benefits and cost effectiveness of testing low-risk groups with no known exposure to SARS-CoV-2, particularly in settings where testing capacity is limited."
- "Suspected COVID-19 cases in outbreak investigations"

The WHO indicates the following as priority uses for the Ag-RDTs:

- "Community testing of symptomatic individuals meeting the case definition of suspected COVID-19."
- "To detect and respond to suspected outbreaks of COVID-19 including in remote settings, institutions and semi-closed communities (e.g., schools, care-homes, cruise ships, prisons, workplaces and dormitories), especially where NAAT is not immediately available."
- "To screen asymptomatic individuals at high risk of COVID-19, including health workers, contacts of cases and other at-risk individuals."

Overall, "Ag-RDT testing is recommended in settings likely to have the most impact on early detection

of cases for care and contact tracing and where test results are most likely to be correct" (WHO, 2021a).

WHO released a second scientific brief with recommendations concerning immunity passports (WHO, 2020b) on April 24, 2020. Within the guidelines, WHO states that as of the publication date, "no study has evaluated whether the presence of antibodies to SARS-CoV-2 confers immunity to subsequent infection by this virus in humans." They go on to note, "Laboratory tests that detect antibodies to SARS-CoV-2 in people, including rapid immunodiagnostic tests, need further validation to determine their accuracy and reliability. Inaccurate immunodiagnostic tests may falsely categorize people in two ways. The first is that they may falsely label people who have been infected as negative, and the second is that people who have not been infected are falsely labelled as positive. Both errors have serious consequences and will affect control efforts. These tests also need to accurately distinguish between past infections from SARS-CoV-2 and those caused by the known set of six human coronaviruses. Four of these viruses cause the common cold and circulate widely. The remaining two are the viruses that cause Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome. People infected by any one of these viruses may produce antibodies that cross-react with antibodies produced in response to infection with SARS-CoV-2 (WHO, 2020b)".

In 2021, WHO released an update to the scientific brief concerning immunity passports within a document titled 'COVID-19 natural immunity.' Within this brief, WHO discusses the various testing methods available. WHO notes that "there are many available serologic assays that measure the antibody response to SARS-CoV-2 infection, but at the present time, the correlates of protection are not well understood". The most measured immune response is the presence of antibodies in serum. Serologic assays to detect the antibody response are usually based on enzyme immunoassays, which detect the presence of virus-specific antibodies in the blood or by live or pseudo-virus neutralization assays, which detect functional NAb. While serologic testing has limited use in clinical management because it does not capture active infection, it can be very useful in determining the extent of infection or estimating attack rates in given populations. Interpreting the results of serologic testing, however, is complex: there are several antibody types and subtypes and multiple antigenic determinants/epitopes that can be used to target these antibodies, and the results may differ substantially depending on the combinations chosen. The results will also depend on the manufacturing specifics of the assay used" (WHO, 2021c). Other frequently used assays are enzyme-linked immunosorbent tests, chemiluminescent tests, and lateral flow rapid diagnostic tests. To conclude, "available tests and current knowledge do not tell us about the duration of immunity and protection against reinfection, but recent evidence suggests that natural infection may provide similar protection against symptomatic disease as vaccination, at least for the available follow up period."

WHO released a scientific brief on May 15, 2020, concerning multisystem inflammatory syndrome in children and adolescents with COVID-19. Within the guidelines, they recommend standardized data describing clinical presentations.

- WHO gives a preliminary case definition for individuals ages 0 – 19 years with fever three or more days AND at least TWO of the following:
  - "Rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs (oral, hands or feet).
  - Hypotension or shock.
  - Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including [echocardiogram] findings or elevated Troponin/NT-proBNP),
  - Evidence of coagulopathy (by PT, PTT, elevated d-Dimers).
  - Acute gastrointestinal problems (diarrhea, vomiting, or abdominal pain).
- AND
  - Elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin.
- AND
  - No other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes.

- AND
  - Evidence of COVID-19 (RT-PCR, antigen test or serology positive), or likely contact with patients with COVID-19 (WHO, 2020c)."

Centers for Disease Control and Prevention (CDC)

In the update of the CDC guidelines *Overview of Testing for SARS-CoV-2*, dated February 11, 2022, the CDC states that "viral tests, including nucleic acid amplification tests (NAATs, such as Reverse Transcription-Polymerase Chain Reaction), antigen tests and other tests (such as breath tests) are used as diagnostic tests to detect current infection with SARS-CoV-2 and to inform an individual's medical care." The CDC also stated, "Viral testing is recommended for individuals who are close contacts of persons with COVID-19" (CDC, 2022g). Regarding use of antibody testing, the CDC remarks: "Antibody testing is not currently recommended to assess a person's protection against infection or severe COVID-19 following COVID-19 vaccination or prior infection, or to assess the need for vaccination in an unvaccinated person. To evaluate for evidence of previous infection in a vaccinated individual, an antibody test specifically evaluating IgM/IgG to the nucleocapsid protein should be used (e.g., for public health surveillance or the diagnosis of Multisystem Inflammatory Syndrome in Children (MIS-C) Multisystem Inflammatory Syndrome in Adults (MIS-A)" (CDC, 2022g).

Regarding testing for asymptomatic patients with "known or suspected exposure to SARS-CoV-2", the CDC recommends testing for all close contacts of persons with SARS-CoV-2 infection. "Viral testing is recommended for individuals who have been exposed persons with COVID-19. People who have had an exposure with someone known or suspected of having COVID-19 should be tested at least 5 days after the exposure. If symptoms develop before 5 days, they should get tested immediately" (CDC, 2022g). The CDC also notes the settings to prioritize for screening testing, including:

- "High-risk congregate settings, such as assisted living facilities, correctional facilities, and homeless shelters, that have demonstrated high potential for rapid and widespread virus transmission to people at high risk for severe illness."
- "Settings that involve close quarters and that are isolated from healthcare resources (e.g., fishing vessels, wildland firefighter camps, or offshore oil platforms)" (CDC, 2022g).

The CDC states it is "working with state, local, territorial, academic, and commercial partners" for surveillance testing and COVID-19 research in the US (CDC, 2022g).

Finally, the CDC still maintains, "Consequently, evidence supports a time-based and symptom-based strategy to determine when to discontinue isolation or other precautions rather than a test-based strategy. For persons who are severely immunocompromised, a test-based strategy could be considered in consultation with infectious disease experts. For all others, a test-based strategy is no longer recommended" (CDC, 2021b).

The CDC also published *Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings*. The CDC states that "Both SARS-CoV-2 IgM and IgG antibodies may be detected around the same time after infection. However, while IgM is most useful for determining recent infection, it usually becomes undetectable weeks to months following infection; in contrast, IgG may remain detectable for longer periods. IgA is important for mucosal immunity and can be detected in mucous secretions like saliva in addition to blood; although, its significance in this disease is still to be determined." The CDC also acknowledges the potential application of neutralizing antibody detection (as opposed to binding antibody detection), and remarks that the FDA has now authorized one competitive neutralization test (cVNT), which is a "binding antibody tests designed to qualitatively detect potentially neutralizing antibodies, often those that prevent interaction of RBD with the ACE-2 receptor" (CDC, 2022f).

According to the interim guidelines, antibody tests can be used to

- "Determine if a person has COVID-19 antibodies, which suggests past infection or vaccination."
- "Aid in the diagnosis of multisystem inflammatory syndrome in children (MIS-C) and in adults (MIS-A)."
- "Monitor and evaluate population levels of immunity."

On the other hand, antibody tests should not be used to

- "Diagnose current infection."
- "Determine if someone can return to work or school."
- "Group people together in settings such as schools, dormitories, and correctional facilities; or to exempt someone from screening testing."
- "Exempt a person who wears personal protective equipment (PPE) at work from following site-specific requirements".

The diagnosis of acute infection from SARS-CoV-2 is best determined by diagnostic testing using a nucleic acid amplification test (NAAT) or antigen test (CDC, 2022e).

Moreover, though CDC asserts that "Antibody testing is not a replacement for virologic testing and should not be used to establish the presence or absence of acute SARS-CoV-2 infection", they also suggest that "Antibody testing may be useful to support the diagnosis of COVID-19 illness or complications of COVID-19 in the following situations:

- A positive antibody test at least 7 days following acute illness onset in persons who had a previous negative antibody test (e.g., seroconversion) but did not receive a positive viral test might indicate SARS-CoV-2 infection between the dates of the negative and positive antibody tests.
- A positive antibody test can help support a diagnosis when patients present with complications of COVID-19, such as multisystem inflammatory syndrome or other post-acute sequelae of COVID-19" (CDC, 2022e).

The CDC also states that "Although current EUA indications do not preclude the use of these tests in vaccinated individuals, none of the currently authorized tests have been specifically authorized to assess immunity or protection of people who have received a COVID-19 vaccine, including people with immunocompromising conditions" (CDC, 2022e).

Regarding testing for past infections, the CDC recommends that antibody tests should not be used to diagnose a current COVID-19 infection (CDC, 2022b).

Within the CDC's *Interim Guidelines for Collecting and Handling Clinical Specimens for COVID-19 Testing*, they recommend collecting and testing upper respiratory samples for initial diagnostic testing for current SARS-CoV-2 infections. Within their recommendation, they list the following (without stating a preference) as acceptable specimens:

- "Nasopharyngeal (NP) specimen collection/oropharyngeal (OP) (throat) specimen collection (performed by a trained healthcare provider, only)
- Nasal mid-turbinate (MT) swab (performed by a healthcare provider or the patient after reviewing and following collection instructions)
- Anterior nasal specimen (performed by a healthcare provider or the patient after reviewing and following collection instructions)
- Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) (performed by a trained healthcare provider)
- Saliva (collected by patient with or without supervision)

- Breath (performed by a qualified, trained operator under the supervision of a healthcare provider licensed or authorized by state law to prescribe tests)
- Bronchoalveolar lavage, tracheal aspirate, pleural fluid, lung biopsy (generally performed by a physician in the hospital setting)
- Sputum (collected under the guidance of a trained healthcare professional)" (CDC, 2022d).

The CDC issued employer-based guidelines titled *Guidance for Businesses and Employers Responding to Coronavirus Disease 2019 (COVID-19): Plan, Prepare, and Respond to Coronavirus Disease 2019*. (CDC, 2021a). This guideline is now considered archived and made available for historical purposes, and the CDC now refers to the OSHA guideline titled *Protecting Workers: Guidance on Mitigating and Preventing the Spread of COVID-19 in the Workplace* for the latest information.

The CDC, in a joint interim set of guidelines with OSHA, issued Meat and Poultry Processing Workers and Employers Interim Guidance from CDC and the Occupational Safety and Health Administration (OSHA) (CDC & OSHA, 2020). This guideline is now considered archived and made available for historical purposes, and the CDC now refers to the OSHA guideline titled *Protecting Workers: Guidance on Mitigating and Preventing the Spread of COVID-19 in the Workplace*.

In their August 31, 2022, update, the CDC recommends a test-based strategy "may be used to remove a mask sooner." The CDC recommends a symptom-based strategy primarily for healthcare professionals. The recommendations are listed below:

- "For people who are mildly ill with SARS-COV-2 infection and not moderately or severely immunocompromised,
  - Isolation can be discontinued at least 5 days after symptom onset (day 0 is the day symptoms appeared, and day 1 is the next full day thereafter) if fever has resolved for at least 24 hours (without taking fever-reducing medications) **and** other symptoms are improving.
  - If symptoms recur or worsen, the isolation period should restart at day 0.
  - People who cannot wear a mask, including children < 2 years of age and people of any age with certain disabilities, should isolate for 10 days.
  - In certain high-risk congregate settings that have high risk of secondary transmission, CDC recommends a 10-day isolation period for residents."
- "For people who test positive, are asymptomatic (never develop symptoms) and not moderately or severely immune compromised,
  - Isolation can be discontinued at least 5 days **after the first positive viral test** (day 0 is the date the specimen was collected for the positive test, and day 1 is the next full day thereafter)."
- "For people who are moderately ill and not moderately or severely immunocompromised, isolation and precautions can be discontinued 10 days after symptom onset (day 0 is the day symptoms appeared, and day 1 is the next full day thereafter)."
- "For people who are severely ill and not moderately or severely immunocompromised, isolation and precautions can be discontinued 10 days after symptom onset (day 0 is the day symptoms appeared, and day 1 is the next full day thereafter).
  - Some people with severe (e.g., requiring hospitalization, intensive care, or ventilation support) may remain infectious beyond 10 days. This may warrant extending the duration of isolation and precautions for up to 20 days after symptom onset (with day 0 being the day symptoms appeared) **and** after resolution of fever for at least 24 hours (without taking fever-reducing medications) **and** improvement of other symptoms.
  - Serial testing prior to ending isolation can be considered in consultation with infectious disease experts."
- "For people who are moderately or severely immunocompromised (regardless of COVID-19 symptoms or severity), … CDC recommends an isolation period of at least 20 days, and ending isolation in conjunction with serial testing and consultation with an infectious disease specialist to determine the appropriate duration of isolation and precautions.

  - The criteria for serial testing to end isolation are:
    - Results are negative from at least two consecutive respiratory specimens collected In certain high-risk congregate settings that have high risk of secondary transmission, CDC recommends a 10-day isolation period for residents.
    - Also, if a moderately or severely immunocompromised patient with COVID-19 was symptomatic, there should be resolution of fever for at least 24 hours (without the taking fever-reducing medication) and improvement of other symptoms" (CDC, 2022c).

In terms of ending isolation for nonhealthcare personnel, the recommendations are below, utilizing a symptom-based strategy as well:

- "If you had no symptoms, you may end isolation after day 5."
- "If you had symptoms and:
  - Your symptoms are improving – you may end isolation after day 5 if you are fever-free for 24 hours (without the use of fever-reducing medication).
  - Your symptoms are not improving – continue to isolate until: you are fever-free for 24 hours (without the use of fever-reducing medication) [and] your symptoms are improving.
- "If you had symptoms and had:
  - Moderate illness (you experienced shortness of breath or had difficulty breathing):
    - You need to isolate through day 10.
  - Severe illness (you were hospitalized) or have a weakened immune system:
    - You need to isolate through day 10.
    - Consult your doctor before ending isolation.
    - Ending isolation without a viral test may not be an option for you" (CDC, 2023b).

The CDC also published a case series of "Multisystem Inflammatory Syndrome in Adults Associated with SARS-CoV-2 Infection" [MIS-A]. The CDC observes that a "hyperinflammatory syndrome resembling MIS-C" may also manifest in adult patients and remarks that "Clinicians and health departments should consider MIS-A in adults with signs and symptoms compatible with the current working MIS-A case definition. Antibody testing for SARS-CoV-2 might be needed to confirm previous COVID-19 infection in patients who do not have positive SARS-CoV-2 PCR or antigen test results." The working case definition of MIS-A was defined by CDC as follows:

- "a severe illness requiring hospitalization in a person aged ≥21 years;
- a positive test result for current or previous SARS-CoV-2 infection (nucleic acid, antigen, or antibody) during admission or in the previous 12 weeks;
- severe dysfunction of one or more extrapulmonary organ systems (e.g., hypotension or shock, cardiac dysfunction, arterial or venous thrombosis or thromboembolism, or acute liver injury);
- laboratory evidence of severe inflammation (e.g., elevated CRP, ferritin, D-dimer, or interleukin-6); and
- absence of severe respiratory illness (to exclude patients in which inflammation and organ dysfunction might be attributable simply to tissue hypoxia)."

Patients with mild respiratory symptoms who met these criteria were included. Patients were excluded if alternative diagnoses such as bacterial sepsis were identified.

The CDC does note three limitations of the case series report, which are as follows:

- "First, cases described here were voluntarily reported or published and therefore are not representative of the true clinical spectrum or racial/ethnic distribution of this emerging syndrome. Additional cases might not have been reported or published; others might have remained unrecognized because of absence of COVID-like symptoms, lack of antibody testing, or negative test results.
- Second, the working case definition excludes patients with severe respiratory dysfunction to

distinguish MIS-A from severe COVID-19; however, the two conditions might overlap in some cases.
- Finally, the working case definition for this syndrome is potentially nonspecific, and some patients with other disease processes might have been misclassified as having MIS-A" (Morris et al., 2020).

On July 9, 2021, the CDC updated their research on "Post-COVID Conditions: Information for Healthcare Providers" (CDC, 2022h). According to the CDC, "Post-COVID conditions" can be referred to as "long COVID, post-acute COVID-19, long-term effects of COVID, post-acute COVID syndrome, chronic COVID, long-haul COVID, late sequelae, and the research term "post-acute sequelae of SARS-COV-2 infection (PASC)." Generally, "it can be considered a lack of return to a usual state of health following acute COVID-19 illness" and "might also include development of new or recurrent symptoms unmasking of a pre-existing condition that occurs after the symptoms of acute illness have resolved." However, the CDC considers post-COVID conditions "to be present if recovery does not occur after the 4-week acute phase even though many patients continue to recover between 4 and 12 weeks." The CDC also warns of the possibility that some patients with post-COVID conditions won't have positive tests for COVID-19 because of "a lack of testing or inaccurate testing during the acute period, or because of waning antibody levels or false-negative antibody testing during follow up." Alternatively, the term "long COVID" is defined as "symptoms lasting 3 or more months that were not present prior to having COVID-19." The CDC cites the most common symptoms to include: "Dyspnea or increased respiratory effort, fatigue, post-exertional malaise and/or poor endurance, cognitive impairment or "brain fog", cough, chest pain, headache, palpitations and tachycardia, arthralgia, myalgia, paresthesia, abdominal pain, diarrhea, insomnia and other sleep difficulties, fever, lightheadedness, impaired daily function and mobility, pain, rash (e.g., urticaria), mood changes, anosmia or dysgeusia, menstrual cycle irregularities, [and] erectile dysfunction." "Post-exertional malaise (PEM)" is defined as "the worsening of symptoms following even minor physical or mental exertion, with symptoms typically worsening 12 to 48 hours after activity and lasting for days or even weeks" (CDC, 2022h).

National Institutes of Health (NIH)

The NIH released COVID-19 treatment guidelines, and within the September 26, 2022, update, they addressed clinical spectrum of SARS-CoV-2 infection, which includes those with asymptomatic or presymptomatic infection, mild illness, moderate illness, severe illness, and critical illness. For asymptomatic and presymptomatic individuals, the NIH states that "It is unclear what percentage of individuals who present with asymptomatic infection progress to clinical disease. Some asymptomatic individuals have been reported to have objective radiographic findings consistent with COVID-19 pneumonia." Additionally, the guideline discusses infectious complications in patients with COVID-19, which can be categorized as "coinfections at presentation," such as "concomitant viral infections, including influenza and respiratory viruses" and community-acquired bacterial pneumonia, and "reactivation of latent infections," such as chronic hepatitis B virus and latent tuberculosis reactivation, "nosocomial infections," such as hospital-acquired or ventilator-associated pneumonia and *Clostridioides difficile*-associated diarrhea, and "opportunistic fungal infections," like aspergillosis and mucormycosis among hospitalized COVID-19 patients (NIH, 2023a).

The NIH also released COVID-19 testing guidelines. The following recommendations were made from the COVID-19 Treatment Guidelines Panel:

- The Panel recommends "using either a nucleic acid amplification test (NAAT) or an antigen test with a sample collected from the upper respiratory tract (e.g., nasopharyngeal, nasal mid-turbinate, or anterior nasal) to diagnose acute infection of SARS-CoV-2 (AIII)."
- "A NAAT should not be repeated in an asymptomatic person (with the exception of health care workers) within 90 days of a previous SARS-CoV-2 infection, even if the person has had a significant exposure to SARS-CoV-2."

- "SARS-CoV-2 reinfection has been reported in people after an initial diagnosis of the infection; therefore, clinicians should consider using a NAAT for those who have recovered from a previous infection and who present with symptoms that are compatible with SARS-CoV-2 infection if there is no alternative diagnosis (BIII)."
- "The Panel **recommends against** diagnosing acute SARS-CoV-2 infection solely on the basis of serologic (i.e., antibody) test results (AIII)."
- "There is insufficient evidence for the Panel to recommend either for or against the use of SARS-CoV-2 serologic testing to assess for immunity or to guide clinical decisions about using COVID-19 vaccines or anti-SARS-CoV-2 monoclonal antibodies" (NIH, 2023b).

American Medical Association (AMA)

The AMA released public health guidelines and recommendations concerning serological testing for SARS-CoV-2 antibodies on May 14, 2020. They list the limitations of antibody testing to include the potential for false-positive results, potential cross-reactivity, and lack of knowledge concerning relationship between antibody testing and immune status. The AMA recommends the following:

- "Use of serology tests should currently be limited to population-level seroprevalence study, evaluation of recovered individuals for convalescent plasma donations, and in other situations where they are used as part of a well-defined testing plan and in concert with other clinical information by physicians well-versed in interpretation of serology test results."
- "Serology tests should not be offered to individuals as a method of determining immune status."
- "Serology tests should not currently be used as the basis for any "immunity certificates," to inform decisions to return to work, or to otherwise inform physical distancing decisions. Doing so may put individuals, their household and their community at risk."
- "Serology tests should not be used as the sole basis of diagnosis of COVID-19 infection" (AMA, 2020).

  "Messaging on serological testing to medically underserved communities should explicitly take into consideration cultural and social features which may bear on their ability to make long-term choices on physical distancing and other COVID-19 precautions" (AMA, 2020).

Infectious Diseases Society of America (IDSA)

The Infectious Diseases Society of America (IDSA) on May 6, 2020, released their guidelines on the diagnosis of COVID-19. At this time, they focus solely on the use of targeted nucleic acid testing, such as RT-PCR, because "[a]t the time of this review, there was little evidence to inform use of serologic testing" (IDSA, 2020b). The IDSA convened a multidisciplinary panel of experts to review the research and literature on the available diagnostic testing for COVID-19. The panel used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology to assess the evidence of the studies and to make their recommendations. A primary recommendation implies that diagnostic testing and specimen collection devices are available whereas a contingency recommendation is made for situations where testing and/or personal protective equipment (PPE) are limited.

The panel made 17 recommendations concerning the use of nucleic acid testing as follows (IDSA, 2020b):

- They **strongly** recommend using a nucleic acid amplification test (NAAT), such as RT-PCR, in symptomatic patients even when clinical suspicion for COVID-19 is low.
- They suggest **(conditional recommendation)** using a nasopharyngeal, mid-turbinate, anterior nasal swab, saliva, or a combined anterior nasal/oropharyngeal swab rather than oropharyngeal swab or saliva sample for testing in symptomatic individuals suspected of COVID-19.
- They suggest **(conditional recommendation**) that either a patient or a healthcare provider can collect an anterior nasal or mid-turbinate sample in a symptomatic patient with upper respiratory

tract infections or influenza-like illness suspected of COVID-19.

- They suggest **(conditional recommendation)** "initially obtaining an upper respiratory tract sample (e.g., nasopharyngeal swab) rather than a lower respiratory sample for SARS-CoV-2 RNA testing in hospitalized patients with suspected COVID-19 lower respiratory tract in infection.
    - If the initial upper respiratory sample result is negative, and the suspicion for disease remains high, the IDSA panel suggests collecting a lower respiratory tract sample (e.g., sputum, bronchoalveolar lavage fluid, tracheal aspirate) rather than collecting another upper respiratory sample."
- They suggest **(conditional recommendation)** performing only one test in a symptomatic individual and not repeat testing if low clinical suspicion of COVID-19.
- They suggest **(conditional recommendation**) repeat testing of an initial negative result in a symptomatic individual be performed only if there is an intermediate or high clinical suspicion of COVID-19.
- They suggest **(conditional recommendation)** "using either rapid RT-PCR or standard laboratory-based NAATs over rapid isothermal NAAT in symptomatic individuals suspected of having COVID-19)."
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals who are either known or suspected to have been exposed to COVID-19.
- They suggest against **(conditional recommendation)** RNA testing in asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with low prevalence. They consider a low prevalence rate to be less than 2% of the community.
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with high prevalence of the disease. They consider a high prevalence rate to be 10% or higher. The IDSA does note that if the prevalence rate is between 2% and 9% the decision to test should be dependent on the availability of testing resources.
- They **strongly** recommend RNA testing in immunocompromised asymptomatic individuals who are being admitted to the hospital regardless of exposure to COVID-19.
- They **strongly** recommend RNA testing (*versus* no testing) in asymptomatic individuals before immunosuppressive procedures, such as a hematopoietic stem cell (HSCT) or solid organ (SOT) transplant regardless of a known exposure to COVID-19.
- They make **NO** recommendations "for or against SARS-CoV-2 RNA testing before initiating immunosuppressive therapy in asymptomatic individuals with cancer," citing an evidence gap. This recommendation does not apply to candidates or recipients of HSCT or SOT.
- They make **NO** recommendations "for or against SARS-CoV-2 RNA testing before the initiation of immunosuppressive therapy in asymptomatic individuals with autoimmune disease," citing an evidence gap.
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals without known exposure to COVID-19 who are undergoing major time-sensitive surgeries.
- They suggest against **(conditional recommendation)** RNA testing in asymptomatic individuals without known exposure to COVID-19 who are undergoing time-sensitive aerosol-generating procedures, such as a bronchoscopy, when PPE is available.
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals without known exposure to COVID-19 who are undergoing time-sensitive aerosol-generating procedures when PPE is limited and testing is available. For this recommendation, the IDSA gives greater detail due to restrictions in availability of PPE. They also note that their recommendation does not address the need for repeat testing if patients require multiple procedures over time.

Besides the 17 recommendations, the IDSA panel also released their algorithm for SARS-CoV-2 Nucleic Acid Testing. This algorithm, as seen in **Figure 2**, separates individuals into symptomatic and asymptomatic groups. The IDSA notes that testing should be prioritized for symptomatic patients first. When resources are sufficient, then testing for selected asymptomatic individuals can be considered. Regardless, the preferred testing methodology is direct SARS-CoV-2 nucleic acid amplification testing,

such as RT-PCR.

Figure 1. IDSA Algorithm for SARS-CoV-2 Nucleic Acid Testing

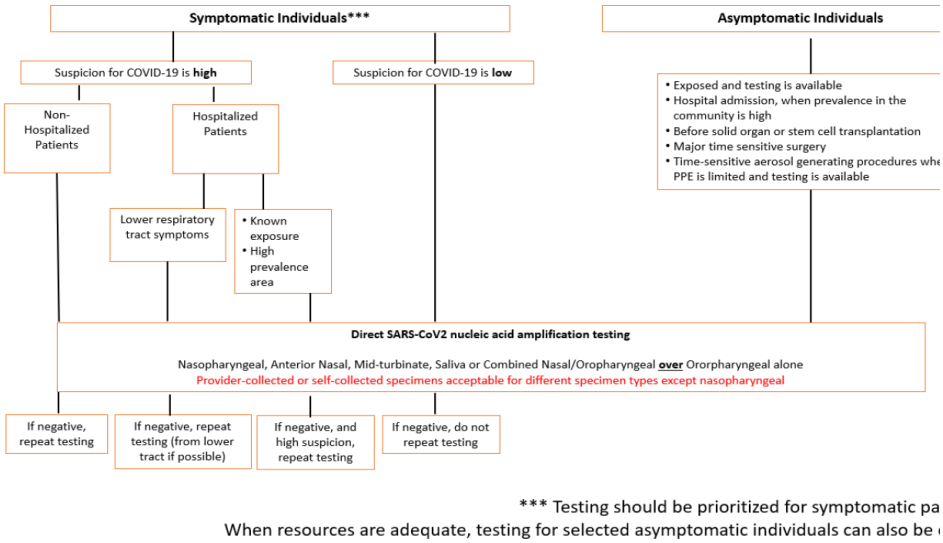

Figure 2: *IDSA Algorithm for SARS-CoV-2 Nucleic Acid Testing* (IDSA, 2020a). The Infectious Diseases Society of America (IDSA) released their algorithm for nucleic acid testing for COVID-19. According to the IDSA guidelines, testing priority should first be given to symptomatic patients; if resources are available, then testing asymptomatic individuals can be considered. Regardless, patients undergoing time-sensitive immunosuppressive procedures should be tested (IDSA, 2020b).

IDSA also published a guideline regarding serology testing on August 18, 2020. In it, they make the following recommendations:

- "The IDSA panel suggests against using serologic testing to diagnose SARS-CoV-2 infection during the first two weeks (14 days) following symptom onset (conditional recommendation, very low certainty of evidence)."
- "When SARS-CoV-2 infection requires laboratory confirmation for clinical or epidemiological purposes, the IDSA panel suggests testing for SARS-CoV-2 IgG or total antibody three to four weeks after symptom onset to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence)."
- The IDSA panel makes no recommendation either for or against using IgM antibodies to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence).
- "The IDSA panel suggests against using IgA antibodies to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence)."
- "The IDSA panel suggests against using IgM or IgG antibody combination tests to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence)."
- "The IDSA panel suggests using IgG antibody to provide evidence of COVID-19 infection in symptomatic patients with a high clinical suspicion and repeatedly negative NAAT testing (weak recommendation, very low certainty of evidence)."
- "In pediatric patients with multisystem inflammatory syndrome, the IDSA panel suggests using both IgG antibody and NAAT to provide evidence of current or past COVID-19 infection (strong recommendation, very low certainty of evidence)."

- "The IDSA panel makes no recommendation for or against using capillary versus venous blood for serologic testing to detect SARS-CoV-2 antibodies (knowledge gap)."

IDSA also includes several comments on testing methodologies that are currently under evaluation. The methodologies named in this section of the guideline are "neutralizing antibody and cellular immune responses", detection of viral antigens aside from the S or N protein, and multi-test algorithms (IDSA, 2020c).

In the 2018 IDSA guidelines, released before the COVID-19 pandemic, IDSA notes, "Suspected cases of SARS coronavirus and MERS coronavirus require immediate notification to the laboratory. Guidance for testing can be found at [CDC websites for SARS and MERS]." For the four endemic human coronaviruses, they only state that they are associated with common cold "symptoms of rhinorrhea, congestion, sore throat, sneezing, and cough and may present with fever". They do note that for children with asthma or otitis media, these viruses can cause exacerbation of the conditions. IDSA notes, "Diagnostic tests include NAATs, which are now common in commercial respiratory panels." Within their table for the laboratory diagnosis of bronchiolitis, bronchitis, and pertussis, the IDSA lists possible diagnostic procedures for the detection of coronavirus to include NAAT, rapid antigen detection tests, and virus culture; however, they do not list one methodology as a preferred or recommended method over another. For the antigen testing, they do include a footnote stating, "Rapid antigen tests for respiratory virus detection lack sensitivity and depending upon the product, specificity" (Miller et al., 2018).

Infectious Diseases Society of America (IDSA)/American Society for Microbiology (ASM)

In 2022, IDSA and ASM released a consensus review document on the clinical and infection prevention applications for SARS-CoV-2 genotyping. In it, they cover clinical use cases for genotyping, methods of genotyping, assay validation and regulatory requirements, clinical reporting for laboratories, and emerging issues in clinical SARS-CoV-2 sequencing. Overall, they report that "while clinical uses of SARS-CoV2 genotyping are currently limited, rapid technological change along with a growing ability to interpret variants in real time foretell a growing role for SARS-CoV-2 genotyping in clinical care as continuing data emerge on vaccine and therapeutic efficacy" (Greninger et al., 2022).

Society for Healthcare Epidemiology of America- (SHEA)/American Society of Anesthesiologists (ASA)/Anesthesia Patient Safety Foundation (APSF)

In late 2022, SHEA published recommendations on screening for SARS-CoV-2 in an asymptomatic population. Here, they note that testing of asymptomatic patients was an attempt to reduce the risk of nosocomial transmission but has been an extensive and resource intensive process with unclear benefit when added to other layers of infection prevention mitigation controls. They also note that "the logistic challenges and costs related to screening program implementation, data noting the lack of substantial aerosol generation with elective controlled intubation, extubation, and other procedures, and the adverse patient and facility consequences of asymptomatic screening call into question the utility of this infection prevention intervention." Based on their findings, SHEA "recommends against routine universal use of asymptomatic screening for SARS-CoV-2 in healthcare facilities. Specifically, preprocedure asymptomatic screening is unlikely to provide incremental benefit in preventing SARS-CoV-2 transmission in the procedural and perioperative environment when other infection prevention strategies are in place, and it should not be considered a requirement for all patients. Admission screening may be beneficial during times of increased virus transmission in some settings where other layers of controls are limited (eg, behavioral health, congregate care, or shared patient rooms), but widespread routine use of admission asymptomatic screening is not recommended over strengthening other infection prevention controls" (Talbot et al., 2023).

This statement is supported by the ASA and the APSF. They specifically note that the "SHEA recommendations provide a rationale for considering a move away from universal screening. Such a

change considers the potential adverse consequences of testing for SARS-CoV-2 in asymptomatic patients. Moreover, we recommend that each facility develop a risk/benefit analysis that includes local/facility infection prevention assessment (e.g., patient population, facility physical layout, and community incidence and transmission of COVID-19 as defined in the SHEA Board Commentary), and a robust system of controls and interventions to prevent virus transmission ("Swiss Cheese" model). The recommendations by SHEA should be considered along with these updated recommendations to operationalize a robust and safe perioperative screening and targeted testing program for the benefit of our patients, our healthcare workers, other hospital patients and the public" (ASA & APSF, 2022).

American Association for Clinical Chemistry (AACC)

The AACC released a set of recommendations for "implementing and interpreting SARS-CoV-2 EUA and LDT [laboratory developed test] serologic testing in clinical laboratories." Serologic testing is currently only used for serum, plasma, and "less frequently, whole-blood or dried blood spots," but not for other sample types, like saliva and cerebrospinal fluid. Serologic testing is "not recommended as the primary approach for diagnosis of SARS-CoV-2 infection." For the recommended use of serologic testing, the AACC stated the following:

- "Serologic testing may be offered as an approach to support diagnosis of COVID -19 illness in symptomatic patients and late phase negative molecular testing or for patients presenting with late complications such as multisystem inflammatory syndrome in children (MIS -C).
- Serologic testing can help identify people who may have been infected with or have recovered from the SARS -CoV -2 infection.
- Serologic testing can be used to screen potential convalescent plasma donors and in the manufacture of convalescent plasma.
- Serologic testing can be used for epidemiology and seroprevalence studies.
- Serologic testing can be used for vaccine response and efficacy studies."

Regarding serologic testing limitations, the AACC stated the following:

- "False positive results may occur.
- Negative results do not preclude acute SARS CoV-2 infection or viral shedding.
- Serologic tests may not differentiate between natural infection and vaccine response.
- Serologic results should not be used for
    - Determining individual protective immunity
    - Return to work decisions
    - Cohorting individuals in congregate settings
    - Assessment of convalescent plasma recipients
    - Use of Personal Protective Equipment
    - Placement of high-risk job functions" (Zhang et al., 2021).

European Centre for Disease Prevention and Control (ECDC)

The ECDC in their guidance for laboratory support in the EU/EEA recommends using WHO-recommended testing strategies for the diagnosis and confirmation of COVID-19 (ECDC, 2022a).

In the ECDC's guideline titled "COVID-19 testing strategies and objectives", the ECDC recommends performing laboratory testing in accordance with the WHO case definition. The following populations should be tested (ECDC, 2022b):

- "Ideally, all people with COVID-19 symptoms should be tested as soon as possible after symptom onset. This requires easy access to testing for all, including non-residents. Test result turnaround time should be minimized, people testing positive should isolate and timely contact tracing should be carried out, ensuring that all close contacts are tested, irrespective of symptoms.

- All patients with acute respiratory symptoms in hospitals and in other healthcare settings, and all specimens from sentinel primary care surveillance should be tested for both SARS-CoV-2 and influenza during the influenza season to monitor incidence and trends over time.
- Healthcare and social care settings require intensive testing when there is documented community transmission. Periodic and comprehensive testing of all staff and residents/patients is recommended to prevent nosocomial transmission. Furthermore, all patients/residents should be tested upon or just prior to admission.
- Clusters or outbreaks may occur in certain settings, such as workplaces, educational facilities, prisons, and migrant detention centres. Testing policies and systems should be in place for rapid detection and control to protect the relevant populations in these settings and to protect the community from amplified transmission.
- Countries experiencing high SARS-CoV-2 transmission in a local community should consider testing the whole population of the affected area. This would enable identification of infectious COVID-19 cases and allow for their prompt isolation to interrupt chains of transmission. Depending on the epidemiological situation, size and population density of the affected area, such an approach could be less disruptive for society than having to introduce and ensure compliance with more stringent public health measures.
- To prevent re-introduction, countries or subnational areas that achieved sustained control of the circulation of SARS-CoV-2 should, in addition to quarantine measures, consider targeted testing and follow-up of individuals coming from other areas within the same country, or from other countries that have not yet achieved sustained control of the virus" (ECDC, 2022b).

Finally, ECDC notes that "Genomic surveillance of SARS-CoV-2 is essential to detect, monitor and assess virus variants that can result in increased transmissibility, disease severity, or have other adverse effects on public health and social control measures. Obtaining timely and accurate information on the emergence and circulation of variants of concern (VOCs) and variants of interest (VOIs) requires robust surveillance systems, including integrated genome sequencing with a well-defined sampling and sequencing strategy to ensure representativeness and reliability of findings" (ECDC, 2021, 2022b).

American Academy of Pediatrics (AAP)

The AAP lists the most common scenarios for testing as symptomatic patients; patients who are asymptomatic but had exposure to a person with confirmed or probable COVID-19 infection; and patients who required screening as part of local public health, school, or workplace requirement. The AAP notes that a person's vaccination status may be a factor in decision-making concerning the need for screening. Additionally, the AAP says that for patients who have symptoms, both NAATs (such as PCR testing) and antigen tests can be used. A positive result indicates a SARS-CoV-2 infection on either PCR or antigen diagnostics. That said, for a patient with a negative antigen result, a provider may repeat the antigen test at 48 hours per FDA guidance.

For purposes of testing symptomatic children who have recently had confirmed infections within 3 months, the AAP says providers should consider the possibility of a false-positive result. Especially using PCR tests and other NAAT tests, as these may remain positive from deposited viral genetic material for several months after an active infection. The AAP notes, "In a child with known exposure and compatible symptoms, there may be situations in which it is reasonable to retest within the 90-day window. If testing is performed within that window, antigen testing is generally preferable to NAATs because of the potential for positive NAAT results attributable to prior infection" (AAP, 2022).

Further, the AAP previously stated in 2020-2021 guidance that antibody (serologic) tests "can provide evidence of previous infection with SARS-CoV-2 but are not useful for the diagnosis of acute infection. A positive antibody test result does not prove that a patient has protection against SARS-CoV-2, although the FDA and vaccine companies use serologic testing as a marker for immunogenicity and protection from SARS-CoV-2 infection. Thus, these tests should not be used to make decisions on grouping people

in classrooms or other facilities at this time, and individuals with positive antibody tests should continue to adhere to guidelines about masking, social distancing, and other preventive measures" (AAP, 2022). The AAP has also included some comments and discussion on Multisystem Inflammatory Syndrome in Children (MIS-C). MIS-C has been observed to have some association with COVID-19, and patients with this syndrome have been observed to test positive "far more often" for past SARS-CoV-2 infection (i.e., antibody testing) than acute infection (RT-PCR or antigen test). The Council of State and Territorial Epidemiologists (CSTE) and CDC defines an MIS-C case by the following criteria:

"An individual aged <21 years and in the absence of a more likely alternative diagnosis:

- Subjective or documented fever (T >38.0° C)
- Clinical severity requiring hospitalization or resulting in death
- C-reactive protein (CRP) >3.0 mg/dL
- New onset manifestations of >2 of the following categories:
  - Cardiac: coronary artery dilatation/aneurysm, left ventricular ejection fraction <55%, or troponin elevated above normal
  - Shock
  - Mucocutaneous: rash, oral mucosal inflammation, conjunctivitis/conjunctival injection or extremity findings (erythema, edema)
  - Gastrointestinal: abdominal pain, vomiting or diarrhea
  - Hematologic: platelet count <150,000/µL, absolute lymphocyte count <1000/µL
- Detection of SARS-CoV-2 nucleic acid/antigen up to 60 days prior to or during hospitalization or in a postmortem specimen, OR detection of antibody associated with current illness, OR close contact with a confirmed/probable COVID-19 case in the 60 days prior to hospitalization" (AAP, 2023).

The CDC delineates a testing algorithm for MIS-C in the outpatient or emergency department setting as follows:

- "Evaluate a child with persistent fever (≥3 days) who is moderately to severely ill with clinical signs of organ dysfunction (eg, gastrointestinal, respiratory, cardiac, mucocutaneous or hematologic). Initial evaluation should include measurement of vital signs, assessment of perfusion and oxygen saturation. Early consultation and coordination with the nearest pediatric infectious disease and rheumatology specialist and pediatric referral center for optimal testing and management should be considered. Laboratory screening for systemic inflammation may be considered and initial lab screenings may include complete blood cell count (CBC) with differential, urine analysis, ESR, and CRP, with the addition of ferritin, LDH, comprehensive metabolic panel, pro-BNP, troponin and fibrinogen depending on initial clinical suspicion and/or evidence of inflammation on initial lab screening. Note that none of these laboratory studies is specific for the diagnosis of MIS-C, so even if there is evidence of significant systemic inflammation, alternative diagnoses must still be considered (eg, pyelonephritis, appendicitis)" (AAP, 2023).

For the evaluation of severely ill appearing or hemodynamically fragile patients, they propose that

> "Severely ill-appearing patients and those in compensated shock or shock should be evaluated and treated in the emergency department/critical care setting. Transfer to a referral center should be arranged. Laboratory tests, as described above, should be performed for initial evaluation regardless of duration of fever. Consultation with pediatric subspecialists (infectious diseases, cardiology, rheumatology) at a local or regional pediatric referral center should be initiated but should not delay transfer to a referral center" (AAP, 2023).

Testing for hospitalized children is delineated below.

"Any child sick enough to warrant admission for fever, abdominal pain, diarrhea and/or organ dysfunction in whom MIS-C is suspected should be cared for in a hospital with tertiary pediatric/cardiac

intensive care units. Although decisions about additional testing will be made by the multidisciplinary team managing the patient, pediatricians can prepare families for an expanded laboratory and cardiac workup that may include:

- Chest radiograph, EKG and troponin. If any of these or physical examination is abnormal, then consult with pediatric cardiology and consider additional diagnostic testing for myocardial injury (echocardiogram and/or cardiac MRI).
- Expanded laboratory tests including pro-BNP, triglycerides, creatine kinase, amylase, blood and urine culture, D-dimer, prothrombin time/partial thromboplastin time (PT/PTT), INR, CRP, ferritin, LDH, comprehensive metabolic panel and fibrinogen, if not already conducted.
- In all cases, COVID-19 testing should be performed with RT-PCR assay and serologic testing. Later serology may be needed if all are negative initially. Serologic tests must be sent prior to administration of intravenous immunoglobulin (IVIG)" (AAP, 2023).

American College of Rheumatology (ACR)
The ACR published guidance regarding MIS-C associated with COVID-19. In it, they list SARS-CoV-2 IgG, IgM, and IgA as part of the diagnostic pathway for MIS-C (Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, MB, et al., 2020).
In a December 5, 2020 update of the above guidelines, the ACR states that ESR, CRP, and testing for SARS-CoV-2 (by PCR or serology) should be considered a "tier 1" (first-line evaluation) for MIS-C (Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, Son, et al., 2020).

In a February 3, 2022 update of the above guideline, the ACR added new information concerning immunomodulatory treatment in MIS-C, hyperinflammation in COVID-19, as well as statements on thrombotic risk and anticoagulation in MIS-C (Henderson et al., 2022).
State and Federal Regulations, as applicable

The FDA issued an "Immediately in Effect Guidance on policy for diagnostics testing in laboratories certified to perform high complexity testing under CLIA prior to Emergency Use Authorization for Coronavirus Disease-2019 during the public health emergency" in February 2020 (FDA, 2022c). This policy was updated on 05/11/2020 to state that the "policy is intended to remain in effect only for the duration of the public health emergency related to COVID-19 declared by the Secretary of Health and Human Services (HHS) on January 31, 2020, effective January 27, 2020, including any renewals made by the HHS Secretary in accordance with section 319(a)(2) of the Public Health Service Act (PHS Act) (FDA, 2022e)." As of October 15, 2021, the FDA had issued 418 different EUAs for COVID-19 testing for either *in vitro* diagnostic products (which includes testing such as point-of-care tests, antibody testing, and antigen testing) or high complexity molecular-based laboratory developed tests (FDA, 2021a).

Moreover, within the HR 748, passed as the CARES Act (or Coronavirus Aid, Relief, and Economic Security Act) as public law 116-136 on March 27, 2020, there are sections concerning coverage and pricing of diagnostic testing for COVID-19 (US, 2020).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 86318, 86328, 86408, 86409, 86413, 86769, 87426, 87428, 87631, 87632, 87633, 87635, 87798, 87811, 87913, 0115U, 0202U, 0223U, 0224U, 0225U, 0226U, 0408U, C9083, U0001, and U0002.

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

AAP. (2022, February 28). COVID-19 Testing Guidance. https://services.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/clinical-guidance/covid-19-testing-guidance/

AAP. (2023, February 8). Multisystem Inflammatory Syndrome in Children (MIS-C) Interim Guidance https://www.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/clinical-guidance/multisystem-inflammatory-syndrome-in-children-mis-c-interim-guidance/

AMA. (2020, 05/14/2020). Serological testing for SARS-CoV-2 antibodies. American Medical Association. Retrieved 05/19/2020 from https://www.ama-assn.org/delivering-care/public-health/serological-testing-sars-cov-2-antibodies

ASA, & APSF. (2022, 12-21-2022). ASA and APSF Updated Statement on Perioperative Testing for SARS-CoV-2 in the Asymptomatic Patient. https://www.apsf.org/news-updates/asa-and-apsf-updated-statement-on-perioperative-testing-for-sars-cov-2-in-the-asymptomatic-patient/

Backer, J. A., Klinkenberg, D., & Wallinga, J. (2020). Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020. Euro Surveill, 25(5). https://doi.org/10.2807/1560-7917.Es.2020.25.5.2000062

Baum, S. G. (2020). Adult Multisystem Inflammatory Syndrome Associated with COVID-19. NEJM. https://www.jwatch.org/na52622/2020/10/21/adult-multisystem-inflammatory-syndrome-associated-with

BD_Veritor. (2020). Veritor™ System https://www.fda.gov/media/139755/download

Bezerra, M. F., Machado, L. C., De Carvalho, V., Docena, C., Brandão-Filho, S. P., Ayres, C. F. J., Paiva, M. H. S., & Wallau, G. L. (2021). A Sanger-based approach for scaling up screening of SARS-CoV-2 variants of interest and concern. Infect Genet Evol, 92, 104910. https://doi.org/10.1016/j.meegid.2021.104910

BioFire. (2020, 05/2020). BioFire® Respiratory Panel 2.1 (RP2.1). FDA. Retrieved 05/04/2020 from https://www.fda.gov/media/137583/download

BioGerm. (2020). 2019-nCoV nucleic acid detection kit. http://www.bio-germ.com/zt.php?class_id=102

BioSpace. (2020, 8/20/20). Quidel to Update Packaging of Point-of-Care Sofia® SARS Antigen Test for COVID-19 to Include Either Nasal or Nasopharyngeal Swabs.

BodiTechMed. (2020). AFIAS COVID-19 Ab. http://www.boditech.co.kr/eng/board/news/board_view.asp?num=30109

Caturegli, G., Materi, J., Howard, B. M., & Caturegli, P. (2020). Clinical Validity of Serum Antibodies to SARS-CoV-2 : A Case-Control Study. Ann Intern Med, 173(8), 614-622. https://doi.org/10.7326/m20-2889

CDC. (2016, February 11). What is whole genome sequencing (WGS)? https://www.cdc.gov/pulsenet/pathogens/wgs.html

CDC. (2020a, 03/15/2020). CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel FDA. Retrieved 04/30/2020 from https://www.fda.gov/media/134922/download

CDC. (2020b, 02/15/2020). Human Coronavirus Types. CDC. Retrieved 05/15/2020 from https://www.cdc.gov/coronavirus/types.html

CDC. (2020c, 7/2/2020). Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay. https://www.fda.gov/media/139744/download

CDC. (2020d, 05/14/2020). Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19). CDC. Retrieved 05/26/2020 from https://emergency.cdc.gov/han/2020/han00432.asp

CDC. (2020e, 12/22/20). Symptoms of Coronavirus. Retrieved 2/11/21 from https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

CDC. (2021a, March 8). Guidance for Businesses and Employers: Plan, Prepare, and Respond to Coronavirus Disease 2019. Centers for Disease Control and Prevention. Retrieved 8/21/20 from https://www.cdc.gov/coronavirus/2019-ncov/community/guidance-business-response.html

CDC. (2021b, October 7). Interim Guidance for SARS-CoV-2 Testing in Non-Healthcare Workplaces. https://www.cdc.gov/coronavirus/2019-ncov/community/organizations/testing-non-healthcare-workplaces.html

CDC. (2021c, 5/20/21). Multisystem Inflammatory Syndrome. CDC. Retrieved 04/19/2022 from https://www.cdc.gov/mis/mis-c/hcp/index.html

CDC. (2022a, 11/14/2022). CDC's Influenza SARS-CoV-2 Multiplex Assay. Centers for Disease Control and Prevention. Retrieved 08/21/20 from https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

CDC. (2022b, 09/28/2022). COVID-19 Testing: What You Need To Know. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/testing.html

CDC. (2022c, September 14). Ending Isolation and Precautions for People with COVID-19: Interim Guidance. CDC. Retrieved 08/18/2020 from https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-in-home-patients.html

CDC. (2022d, July 15). Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

CDC. (2022e, Jan. 24, 2022). Interim Guidelines for COVID-19 Antibody Testing. Retrieved 04/20/2022 from https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html

CDC. (2022f, January 24). Interim Guidelines for COVID-19 Antibody Testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html

CDC. (2022g, September 28). Overview of Testing for SARS-CoV-2, the virus that causes COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html

CDC. (2022h, 12/16/2022). Post-COVID Conditions: Information for Healthcare Providers. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/post-covid-conditions.html

CDC. (2023a, 03/16/2023). Interim Clinical Considerations for Use of COVID-19 Vaccines Currently Approved or Authorized in the United States. https://www.cdc.gov/vaccines/covid-19/clinical-considerations/interim-considerations-us.html

CDC. (2023b, 03/21/2023). Isolations and Precautions for People with COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/your-health/isolation.html

CDC. (2023c, March 20). SARS-CoV-2 Variant Classifications and Definitions. Retrieved April 19, 2022 from https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html

CDC, & OSHA. (2020, July 9). Meat and Poultry Processing Workers and Employers: Interim Guidance from CDC and the Occupational Safety and Health Administration (OSHA). Retrieved 08/18/2020 from https://stacks.cdc.gov/view/cdc/90395

Cevik, M., Tate, M., Lloyd, O., Maraolo, A. E., Schafers, J., & Ho, A. (2021). SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. The Lancet Microbe, 2(1), E13-E22. https://doi.org/10.1016/S2666-5247(20)30172-5

Chan, J. F., Yip, C. C., To, K. K., Tang, T. H., Wong, S. C., Leung, K. H., Fung, A. Y., Ng, A. C., Zou, Z., Tsoi, H. W., Choi, G. K., Tam, A. R., Cheng, V. C., Chan, K. H., Tsang, O. T., & Yuen, K. Y. (2020). Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens. J Clin Microbiol, 58(5). https://doi.org/10.1128/jcm.00310-20

Chau, N. V. V., Hong, N. T. T., Ngoc, N. M., Anh, N. T., Trieu, H. T., Nhu, L. N. T., Yen, L. M., Minh, N. N. Q., Phong, N. T., Truong, N. T., Huong, L. T. T., Tu, T. N. H., Hung, L. M., Thanh, T. T., Dung, N. T., Dung, N. T., Thwaites, G., Van Tan, L., & for, O. C.-r. g. (2021). Rapid whole-genome sequencing to inform COVID-19 outbreak response in Vietnam. The Journal of infection, 82(6), 276-316. https://doi.org/10.1016/j.jinf.2021.03.017

Churiwal, M., Lin, K. D., Khan, S., Chhetri, S., Muller, M. S., Tompkins, K., Smith, J., Litel, C., Whittelsey, M., Basham, C., Rapp, T., Cerami, C., Premkumar, L., & Lin, J. T. (2021). Assessment of the Field Utility of a Rapid Point-of-Care Test for SARS-CoV-2 Antibodies in a Household Cohort. Am J Trop Med Hyg, 106(1), 156-159. https://doi.org/10.4269/ajtmh.21-0592

Corman, V. M., Lienau, J., & Witzenrath, M. (2019). [Coronaviruses as the cause of respiratory infections]. Internist (Berl), 60(11), 1136-1145. https://doi.org/10.1007/s00108-019-00671-5 (Coronaviren als Ursache respiratorischer Infektionen.)

Cucinotta, D., & Vanelli, M. (2020). WHO Declares COVID-19 a Pandemic. Acta Biomed, 91(1), 157-160. https://doi.org/10.23750/abm.v91i1.9397

Dao Thi, V. L., Herbst, K., Boerner, K., Meurer, M., Kremer, L. P. M., Kirrmaier, D., Freistaedter, A., Papagiannidis, D., Galmozzi, C., Stanifer, M. L., Boulant, S., Klein, S., Chlanda, P., Khalid, D., Barreto Miranda, I., Schnitzler, P., Kräusslich, H.-G., Knop, M., & Anders, S. (2020). A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. Science Translational Medicine, 12(556), eabc7075. https://doi.org/10.1126/scitranslmed.abc7075

DeBiasi, R. L., Song, X., Delaney, M., Bell, M., Smith, K., Pershad, J., Ansusinha, E., Hahn, A., Hamdy, R., Harik, N., Hanisch, B., Jantausch, B., Koay, A., Steinhorn, R., Newman, K., & Wessel, D. (2020). Severe COVID-19 in Children and Young Adults in the Washington, DC Metropolitan Region. J Pediatr. https://doi.org/10.1016/j.jpeds.2020.05.007

Diao, B., Wen, K., Chen, J., Liu, Y., Yuan, Z., Han, C., Chen, J., Pan, Y., Chen, L., Dan, Y., Wang, J., Chen, Y., Deng, G., Zhou, H., & Wu, Y. (2020). Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. medRxiv, 2020.2003.2007.20032524. https://doi.org/10.1101/2020.03.07.20032524

Dighe, K., Moitra, P., Alafeef, M., Gunaseelan, N., & Pan, D. (2022). A rapid RNA extraction-free lateral flow assay for molecular point-of-care detection of SARS-CoV-2 augmented by chemical probes. Biosensors and Bioelectronics, 200, 113900. https://doi.org/https://doi.org/10.1016/j.bios.2021.113900

ECDC. (2021, May 3). Guidance for representative and targeted genomic SARS-CoV-2 monitoring. https://www.ecdc.europa.eu/en/publications-data/guidance-representative-and-targeted-genomic-sars-cov-2-monitoring

ECDC. (2022a, 03/22/2022). Diagnostic testing and screening for SARS-CoV-2. European Centre for Disease Prevention and Control. Retrieved 04/18/2022 from https://www.ecdc.europa.eu/en/covid-19/latest-evidence/diagnostic-testing

ECDC. (2022b, December 15). Testing strategies for SARS-CoV-2. https://www.ecdc.europa.eu/en/covid-19/surveillance/testing-strategies

EpitopeDiagnostics. (2020). EDI™ Novel Coronavirus COVID-19 ELISA Kits. http://www.epitopediagnostics.com/covid-19-elisa

Espejo, A. P., Akgun, Y., Al Mana, A. F., Tjendra, Y., Millan, N. C., Gomez-Fernandez, C., & Cray, C. (2020). Review of Current Advances in Serologic Testing for COVID-19. Am J Clin Pathol, 154(3), 293-304. https://doi.org/10.1093/ajcp/aqaa112

FDA. (2020a). ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 RT-PCR Assay. https://www.fda.gov/media/141192/download

FDA. (2020b, 7/9/2020). CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay. https://www.fda.gov/media/139743/download

FDA. (2020c). Coronavirus (COVID-19) Update: FDA Issues First Emergency Authorization for Sample Pooling in Diagnostic Testing. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-first-emergency-authorization-sample-pooling-diagnostic

FDA. (2021a, May 11). Coronavirus (COVID-19) Update: 10/15/21. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-101521

FDA. (2021b, April 22). Illumina COVIDSeq Test. https://www.fda.gov/media/138778/download

FDA. (2022a, 02/24/2022). Antibody (Serology) Testing for COVID-19: Information for Patients and Consumers. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/antibody-serology-testing-covid-19-information-patients-and-consumers

FDA. (2022b). Coronavirus (COVID-19) Update: FDA Authorizes First COVID-19 Diagnostic Test Using Breath Samples. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-covid-19-diagnostic-test-using-breath-samples

FDA. (2022c, 04/14/2022). Emergency Use Authorization. Retrieved 04/20/2022 from https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

FDA. (2022d, January 29). In Vitro Diagnostics EUAs. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

FDA. (2022e). Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised). FDA. Retrieved 04/20/2022 from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised

GenMark_Diagnostics. (2020). ePlex Respiratory Pathogen Panel 2. https://www.fda.gov/media/142902/download

Greninger, A. L., Dien Bard, J., Colgrove, R. C., Graf, E. H., Hanson, K. E., Hayden, M. K., Humphries, R. M., Lowe, C. F., Miller, M. B., Pillai, D. R., Rhoads, D. D., Yao, J. D., & Lee, F. M. (2022). Clinical and Infection Prevention Applications of Severe Acute Respiratory Syndrome Coronavirus 2 Genotyping: An Infectious Diseases Society of America/American Society for Microbiology Consensus Review Document. Clin Infect Dis, 74(8), 1496-1502. https://doi.org/10.1093/cid/ciab761

Griffin, D. (2020, December 31). Viral Load as a Predictor of COVID-19 Patient Outcomes. https://www.cuimc.columbia.edu/news/viral-load-predictor-covid-19-patient-outcomes

Guo, L., Ren, L., Yang, S., Xiao, M., Chang, D., Yang, F., Dela Cruz, C. S., Wang, Y., Wu, C., Xiao, Y., Zhang, L., Han, L., Dang, S., Xu, Y., Yang, Q.-W., Xu, S.-Y., Zhu, H.-D., Xu, Y.-C., Jin, Q., . . . Wang, J. (2020). Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciaa310

Helix. (2020, 8/6/20). Helix COVID-19 NGS Test. Retrieved 8/20/20 from https://www.fda.gov/media/140917/download

Henderson, L. A., Canna, S. W., Friedman, K. G., Gorelik, M., Lapidus, S. K., Bassiri, H., Behrens, E. M., Ferris, A., Kernan, K. F., Schulert, G. S., Seo, P., MB, F. S., Tremoulet, A. H., Yeung, R. S. M., Mudano, A. S., Turner, A. S., Karp, D. R., & Mehta, J. J. (2020). American College of Rheumatology Clinical Guidance for Multisystem Inflammatory Syndrome in Children Associated With SARS-CoV-2 and Hyperinflammation in Pediatric COVID-19: Version 1. Arthritis Rheumatol. https://doi.org/10.1002/art.41454

Henderson, L. A., Canna, S. W., Friedman, K. G., Gorelik, M., Lapidus, S. K., Bassiri, H., Behrens, E. M., Ferris, A., Kernan, K. F., Schulert, G. S., Seo, P., Son, M. B. F., Tremoulet, A. H., Yeung, R. S. M., Mudano, A. S., Turner, A. S., Karp, D. R., & Mehta, J. J. (2020). American College of Rheumatology Clinical Guidance for Pediatric Patients with Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with SARS-CoV-2 and Hyperinflammation in COVID-19. Version 2. Arthritis Rheumatol. https://doi.org/10.1002/art.41616

Henderson, L. A., Canna, S. W., Friedman, K. G., Gorelik, M., Lapidus, S. K., Bassiri, H., Behrens, E. M., Kernan, K. F., Schulert, G. S., Seo, P., Son, M. B. F., Tremoulet, A. H., VanderPluym, C., Yeung, R. S. M., Mudano, A. S., Turner, A. S., Karp, D. R., & Mehta, J. J. (2022). American College of Rheumatology Clinical Guidance for Multisystem Inflammatory Syndrome in Children Associated With SARS–CoV-2 and Hyperinflammation in Pediatric COVID-19: Version 3. Arthritis & Rheumatology, 74(4), e1-e20. https://doi.org/https://doi.org/10.1002/art.42062

Hirotsu, Y., Maejima, M., Shibusawa, M., Nagakubo, Y., Hosaka, K., Amemiya, K., Sueki, H., Hayakawa, M., Mochizuki, H., Tsutsui, T., Kakizaki, Y., Miyashita, Y., Yagi, S., Kojima, S., & Omata, M. (2020). Comparison of Automated SARS-CoV-2 Antigen Test for COVID-19 Infection with Quantitative RT-PCR using 313 Nasopharyngeal Swabs Including from 7 Serially Followed Patients. Int J Infect Dis. https://doi.org/10.1016/j.ijid.2020.08.029

Hogan, C. A., Sahoo, M. K., & Pinsky, B. A. (2020). Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. Jama, 323(19), 1967-1969. https://doi.org/10.1001/jama.2020.5445

Hulick, P. (2022, August 10). Next-generation DNA sequencing (NGS): Principles and clinical applications. Wolters Kluwer. https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications

IDSA. (2020a, December 23). IDSA Algorithm for SARS-CoV-2 Nucleic Acid Testing. IDSA. Retrieved 05/13/2020 from https://www.idsociety.org/globalassets/idsa/practice-guidelines/covid-19/diagnostics/figure-01.png

IDSA. (2020b, December 23). Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19: Molecular Diagnostic Testing. IDSA. Retrieved 05/13/2020 from https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/

IDSA. (2020c, August 18). Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19: Serologic Testing. https://www.idsociety.org/practice-guideline/covid-19-guideline-serology/

JHU. (2022, November 11). COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). Johns Hopkins University. https://coronavirus.jhu.edu/map.html

Jones, V. G., Mills, M., Suarez, D., Hogan, C. A., Yeh, D., Bradley Segal, J., Nguyen, E. L., Barsh, G. R., Maskatia, S., & Mathew, R. (2020). COVID-19 and Kawasaki Disease: Novel Virus and Novel Case. Hosp Pediatr. https://doi.org/10.1542/hpeds.2020-0123

Kawasuji, H., Takegoshi, Y., Kaneda, M., Ueno, A., Miyajima, Y., Kawago, K., Fukui, Y., Yoshida, Y., Kimura, M., Yamada, H., Sakamaki, I., Tani, H., Morinaga, Y., & Yamamoto, Y. (2020). Transmissibility of COVID-19 depends on the viral load around onset in adult and symptomatic patients. PLOS ONE, 15(12), e0243597. https://doi.org/10.1371/journal.pone.0243597

Ko, J. H., Joo, E. J., Park, S. J., Baek, J. Y., Kim, W. D., Jee, J., Kim, C. J., Jeong, C., Kim, Y. J., Shon, H. J., Kang, E. S., Choi, Y. K., & Peck, K. R. (2020). Neutralizing Antibody Production in Asymptomatic and Mild COVID-19 Patients, in Comparison with Pneumonic COVID-19 Patients. J Clin Med, 9(7). https://doi.org/10.3390/jcm9072268

Kontou, P. I., Braliou, G. G., Dimou, N. L., Nikolopoulos, G., & Bagos, P. G. (2020). Antibody Tests in Detecting SARS-CoV-2 Infection: A Meta-Analysis. Diagnostics (Basel), 10(5). https://doi.org/10.3390/diagnostics10050319

Kweon, O. J., Lim, Y. K., Kim, H. R., Kim, M. C., Choi, S. H., Chung, J. W., & Lee, M. K. (2020). Antibody kinetics and serologic profiles of SARS-CoV-2 infection using two serologic assays. PLOS ONE, 15(10), e0240395. https://doi.org/10.1371/journal.pone.0240395

LabCorp. (2020a, 3/16/20). ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY. Retrieved 8/21/20 from https://www.fda.gov/media/136151/download

LabCorp. (2020b, 04/20/2020). ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY COVID-19 RT-PCR TEST (LABORATORY CORPORATION OF AMERICA). FDA. Retrieved 04/26/2020 from https://www.fda.gov/media/136151/download

Lambert-Niclot, S., Cuffel, A., Le Pape, S., Vauloup-Fellous, C., Morand-Joubert, L., Roque-Afonso, A. M., Le Goff, J., & Delaugerre, C. (2020). Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs. J Clin Microbiol, 58(8). https://doi.org/10.1128/jcm.00977-20

Li, M., Wei, R., Yang, Y., He, T., Shen, Y., Qi, T., Han, T., Song, Z., Zhu, Z., Ma, X., Lin, Y., Yuan, Y., Zhao, K., Lu, H., & Zhou, X. (2021). Comparing SARS-CoV-2 Testing in Anterior Nasal Vestibular Swabs vs. Oropharyngeal Swabs. Front Cell Infect Microbiol, 11, 653794. https://doi.org/10.3389/fcimb.2021.653794

Li, Y., Yao, L., Li, J., Chen, L., Song, Y., Cai, Z., & Yang, C. (2020). Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. Journal of medical virology, 92(7), 903-908. https://doi.org/10.1002/jmv.25786

Lippi, G., Simundic, A. M., & Plebani, M. (2020). Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med. https://doi.org/10.1515/cclm-2020-0285

Lisboa Bastos, M., Tavaziva, G., Abidi, S. K., Campbell, J. R., Haraoui, L. P., Johnston, J. C., Lan, Z., Law, S., MacLean, E., Trajman, A., Menzies, D., Benedetti, A., & Ahmad Khan, F. (2020). Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. Bmj, 370, m2516. https://doi.org/10.1136/bmj.m2516

Loeffelholz, M. J., & Tang, Y.-W. (2020). Laboratory diagnosis of emerging human coronavirus infections – the state of the art. Emerging Microbes & Infections, 9(1), 747-756. https://doi.org/10.1080/22221751.2020.1745095

Lu, Y., Li, L., Ren, S., Liu, X., Zhang, L., Li, W., & Yu, H. (2020). Comparison of the diagnostic efficacy between two PCR test kits for SARS-CoV-2 nucleic acid detection. Journal of Clinical Laboratory Analysis, 34(10), e23554. https://doi.org/10.1002/jcla.23554

Ludwig, S., & Zarbock, A. (2020). Coronaviruses and SARS-CoV-2: A Brief Overview. Anesth Analg. https://doi.org/10.1213/ane.0000000000004845

LumiraDx. (2020). SARS-CoV-2 Ag Test. https://www.fda.gov/media/141304/download

Mak, G. C., Cheng, P. K., Lau, S. S., Wong, K. K., Lau, C. S., Lam, E. T., Chan, R. C., & Tsang, D. N. (2020). Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol, 129, 104500. https://doi.org/10.1016/j.jcv.2020.104500

Mawhorter, M. E., Nguyen, P., Goldsmith, M., Owens, R. G., Baer, B., & Raman, J. D. (2022). Diagnostic yield and costs associated with a routine pre-operative COVID-19 testing algorithm for asymptomatic patients prior to elective surgery. Am J Clin Exp Urol, 10(5), 341-344.

Mboumba Bouassa, R.-S., Tonen-Wolyec, S., Veyer, D., Péré, H., & Bélec, L. (2022). Analytical performances of the AMPLIQUICK® Respiratory Triplex assay for simultaneous detection and differentiation of SARS-CoV-2, influenza A/B and respiratory syncytial viruses in respiratory specimens. PLOS ONE, 17(1), e0262258. https://doi.org/10.1371/journal.pone.0262258

McIntosh, K. (2023, March 23). COVID-19: Clinical features. Wolter Kluwer. Retrieved 08/19/2020 from https://www.uptodate.com/contents/covid-19-clinical-features

Miller, J. M., Binnicker, M. J., Campbell, S., Carroll, K. C., Chapin, K. C., Gilligan, P. H., Gonzalez, M. D., Jerris, R. C., Kehl, S. C., Patel, R., Pritt, B. S., Richter, S. S., Robinson-Dunn, B., Schwartzman, J. D., Snyder, J. W., Telford, S., 3rd, Theel, E. S., Thomson, R. B., Jr., Weinstein, M. P., & Yao, J. D. (2018). A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis, 67(6), e1-e94. https://doi.org/10.1093/cid/ciy381

Morell, A., Skvaril, F., Noseda, G., & Barandun, S. (1973). Metabolic properties of human IgA subclasses. Clinical and experimental immunology, 13(4), 521-528. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1553728/

Morris, S. B., Schwartz, N. G., Patel, P., Abbo, L., Beauchamps, L., Balan, S., Lee, E. H., Paneth-Pollak, R., Geevarughese, A., Lash, M. K., Dorsinville, M. S., Ballen, V., Eiras, D. P., Newton-Cheh, C., Smith, E., Robinson, S., Stogsdill, P., Lim, S., Fox, S. E., . . . Godfred-Cato, S. (2020). Case Series of Multisystem Inflammatory Syndrome in Adults Associated with SARS-CoV-2 Infection - United Kingdom and United States, March-August 2020. MMWR Morb Mortal Wkly Rep, 69(40), 1450-1456. https://doi.org/10.15585/mmwr.mm6940e1

Nackerdien, Z. (2020, December 6). Viral Load Peaks in First Week of COVID-19 Symptom Onset. Retrieved January 31 from https://www.medpagetoday.org/infectiousdisease/covid19/90035

Nagura-Ikeda, M., Imai, K., Tabata, S., Miyoshi, K., Murahara, N., Mizuno, T., Horiuchi, M., Kato, K., Imoto, Y., Iwata, M., Mimura, S., Ito, T., Tamura, K., & Kato, Y. (2020). Clinical evaluation of self-collected saliva by RT-qPCR, direct RT-qPCR, RT-LAMP, and a rapid antigen test to diagnose COVID-19. J Clin Microbiol. https://doi.org/10.1128/jcm.01438-20

NIH. (2023a, March 6). Clinical Spectrum of SARS-CoV-2 Infection. National Institutes of Health. https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/

NIH. (2023b, March 6). Testing for SARS-CoV-2 Infection. National Institutes of Health. https://www.covid19treatmentguidelines.nih.gov/overview/sars-cov-2-testing/

Okba, N. M. A., Müller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., Lamers, M. M., Sikkema, R. S., de Bruin, E., Chandler, F. D., Yazdanpanah, Y., Le Hingrat, Q., Descamps, D., Houhou-Fidouh, N., Reusken, C., Bosch, B. J., Drosten, C., Koopmans, M. P. G., & Haagmans, B. L.

(2020). Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerg Infect Dis, 26(7). https://doi.org/10.3201/eid2607.200841

Oude Munnink, B. B., Nieuwenhuijse, D. F., Stein, M., O'Toole, Á., Haverkate, M., Mollers, M., Kamga, S. K., Schapendonk, C., Pronk, M., Lexmond, P., van der Linden, A., Bestebroer, T., Chestakova, I., Overmars, R. J., van Nieuwkoop, S., Molenkamp, R., van der Eijk, A. A., GeurtsvanKessel, C., Vennema, H., . . . The Dutch-Covid-19 response, t. (2020). Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. Nature Medicine, 26(9), 1405-1410. https://doi.org/10.1038/s41591-020-0997-y

Padoan, A., Cosma, C., Sciacovelli, L., Faggian, D., & Plebani, M. (2020). Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med. https://doi.org/10.1515/cclm-2020-0443

Palavecino, E. (2015). One Sample, Multiple Results The Use of Multiplex PCR for Diagnosis of Infectious Syndromes. Retrieved 11/1 from https://www.aacc.org/publications/cln/articles/2015/april/one-sample-multiple-results

Patel, P., DeCuir, J., Abrams, J., Campbell, A. P., Godfred-Cato, S., & Belay, E. D. (2021). Clinical Characteristics of Multisystem Inflammatory Syndrome in Adults: A Systematic Review. JAMA Network Open, 4(9), e2126456-e2126456. https://doi.org/10.1001/jamanetworkopen.2021.26456

Peacock, W. F., Soto-Ruiz, K. M., House, S. L., Cannon, C. M., Headden, G., Tiffany, B., Motov, S., Merchant-Borna, K., Chang, A. M., Pearson, C., Patterson, B. W., Jones, A. E., Miller, J., Varon, J., Bastani, A., Clark, C., Rafique, Z., Kea, B., Eppensteiner, J., . . . Young, S. (2022). Utility of COVID-19 antigen testing in the emergency department. Journal of the American College of Emergency Physicians Open, 3(1), e12605. https://doi.org/https://doi.org/10.1002/emp2.12605

Pfefferle, S., Reucher, S., Nörz, D., & Lütgehetmann, M. (2020). Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. Euro Surveill, 25(9). https://doi.org/10.2807/1560-7917.Es.2020.25.9.2000152

Poljak, M., Korva, M., Gašper, N. K., Komloš, K. F., Sagadin, M., Uršič, T., Županc, T. A., Petrovec, M., & McAdam, A. J. (2020). Clinical Evaluation of the cobas SARS-CoV-2 Test and a Diagnostic Platform Switch during 48 Hours in the Midst of the COVID-19 Pandemic. Journal of Clinical Microbiology, 58(6), e00599-00520. https://doi.org/doi:10.1128/JCM.00599-20

Poplar. (2020). EMERGENCY USE AUTHORIZATION (EUA) SUMMARY OF THE POPLAR SARS-COV-2 TMA POOLING ASSAY. https://www.fda.gov/media/140792/download

Qiagen_GmbH. (2020, 03/2020). QIAstat-Dx® Respiratory SARS-CoV2 Panel Instructions for Use (Handbook). FDA. Retrieved 04/27/2020 from https://www.fda.gov/media/136571/download

Quidel_Corporation. (2020, 05/2020). Sofia 2 SARS Antigen FIA. FDA. Retrieved 05/12/2020 from https://www.fda.gov/media/137885/download

Ryding, S. (2020, June 24). What is Viral Load? Retrieved January 31 from https://www.news-medical.net/health/What-is-Viral-Load.aspx

SansureBiotech. (2020, 05-04-2020). Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing). https://www.fda.gov/media/137651/download

Scohy, A., Anantharajah, A., Bodéus, M., Kabamba-Mukadi, B., Verroken, A., & Rodriguez-Villalobos, H. (2020). Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol, 129, 104455. https://doi.org/10.1016/j.jcv.2020.104455

Seo, G., Lee, G., Kim, M. J., Baek, S. H., Choi, M., Ku, K. B., Lee, C. S., Jun, S., Park, D., Kim, H. G., Kim, S. J., Lee, J. O., Kim, B. T., Park, E. C., & Kim, S. I. (2020). Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor. ACS Nano, 14(4), 5135-5142. https://doi.org/10.1021/acsnano.0c02823

Sri Santosh, T., Parmar, R., Anand, H., Srikanth, K., & Saritha, M. (2020). A Review of Salivary Diagnostics and Its Potential Implication in Detection of Covid-19. Cureus, 12(4), e7708. https://doi.org/10.7759/cureus.7708

Talbot, T. R., Hayden, M. K., Yokoe, D. S., Malani, A. N., Amer, H. A., Kalu, I. C., Logan, L. K., Moehring, R. W., Munoz-Price, S., Palmore, T. N., Weber, D. J., Wright, S. B., & Trustees, S. B. o. (2023). Asymptomatic screening for severe acute respiratory coronavirus virus 2 (SARS-CoV-2) as an infection prevention measure in healthcare facilities: Challenges and considerations. Infect Control Hosp Epidemiol, 44(1), 2-7. https://doi.org/10.1017/ice.2022.295

Taylor, J., Carter, R. J., Lehnertz, N., Kazazian, L., Sullivan, M., Wang, X., Garfin, J., Diekman, S., Plumb, M., Bennet, M. E., Hale, T., Vallabhaneni, S., Namugenyi, S., Carpenter, D., Turner-Harper, D., Booth, M., Coursey, E. J., Martin, K., McMahon, M., . . . Lynfield, R. (2020). Serial Testing for SARS-CoV-2 and Virus Whole Genome Sequencing Inform Infection Risk at Two Skilled Nursing Facilities with COVID-19 Outbreaks – Minnesota, April-June 2020. MMWR Morb Mortal Wkly Rep, 69(37), 1288-1295. https://doi.org/10.15585/mmwr.mm6937a3

The_Native_Antigen_Company. (2020, 03/24/2020). Why We Need Antigen and Antibody Tests for COVID-19. The Native Antigen Company. Retrieved 04/21/2020 from https://thenativeantigencompany.com/why-we-need-antigen-and-antibody-tests-for-covid-19/

To, K. K. W., Yip, C. C. Y., Lai, C. Y. W., Wong, C. K. H., Ho, D. T. Y., Pang, P. K. P., Ng, A. C. K., Leung, K. H., Poon, R. W. S., Chan, K. H., Cheng, V. C. C., Hung, I. F. N., & Yuen, K. Y. (2019). Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study. Clin Microbiol Infect, 25(3), 372-378. https://doi.org/10.1016/j.cmi.2018.06.009

UCSD. (2020). UCSD RC SARS-CoV-2 Assay https://www.fda.gov/media/140712/download

US. (2020, 03/27/2020). H.R.748 - CARES Act. Retrieved 05/19/2020 from https://www.congress.gov/116/bills/hr748/BILLS-116hr748enr.pdf

Verdoni, L., Mazza, A., Gervasoni, A., Martelli, L., Ruggeri, M., Ciuffreda, M., Bonanomi, E., & D'Antiga, L. (2020). An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. https://doi.org/10.1016/s0140-6736(20)31103-x

Villaverde, S., Domínguez-Rodríguez, S., Sabrido, G., Pérez-Jorge, C., Plata, M., Romero, M. P., Grasa, C. D., Jiménez, A. B., Heras, E., Broncano, A., Núñez, M. D. M., Illán, M., Merino, P., Soto, B., Molina-Arana, D., Bermejo, A., Mendoza, P., Gijón, M., Pérez-Moneo, B., . . . Epidemiological Study of, C.-i. C. o. t. S. S. o. P. W. G. (2021). Diagnostic Accuracy of the Panbio Severe Acute Respiratory Syndrome Coronavirus 2 Antigen Rapid Test Compared with Reverse-Transcriptase Polymerase Chain Reaction Testing of Nasopharyngeal Samples in the Pediatric Population. The Journal of pediatrics, 232, 287-289.e284. https://doi.org/10.1016/j.jpeds.2021.01.027

Wang, F., Huang, S., Gao, R., Zhou, Y., Lai, C., Li, Z., Xian, W., Qian, X., Li, Z., Huang, Y., Tang, Q., Liu, P., Chen, R., Liu, R., Li, X., Tong, X., Zhou, X., Bai, Y., Duan, G., . . . Liu, L. (2020). Initial whole-genome sequencing and analysis of the host genetic contribution to COVID-19 severity and susceptibility. Cell Discovery, 6(1), 83. https://doi.org/10.1038/s41421-020-00231-4

Wang, R., Qian, C., Pang, Y., Li, M., Yang, Y., Ma, H., Zhao, M., Qian, F., Yu, H., Liu, Z., Ni, T., Zheng, Y., & Wang, Y. (2020). opvCRISPR: One-pot visual RT-LAMP-CRISPR platform for SARS-cov-2 detection. Biosensors and Bioelectronics, 172, 112766. https://doi.org/https://doi.org/10.1016/j.bios.2020.112766

WHO. (2020a, 09/11/20). Diagnostic testing for SARS-CoV-2. Retrieved 11/08/20 from https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

WHO. (2020b, 04/24/2020). "Immunity passports" in the context of COVID-19. World Health Organization. Retrieved 04/25/2020 from https://www.who.int/news-room/commentaries/detail/immunity-passports-in-the-context-of-covid-19

WHO. (2020c, 05/15/2020). Multisystem inflammatory syndrome in children and adolescents with COVID-19. World Health Organization. Retrieved 05/18/2020 from https://www.who.int/publications-detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19

WHO. (2021a, October 6). Antigen-detection in the diagnosis of SARS-CoV-2 infection. World Health Organization. Retrieved 11/08/2020 from https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays

WHO. (2021b, November 2021). COVID-19 Clinical management: living guidance. World Health Organization. Retrieved April 19 from https://apps.who.int/iris/bitstream/handle/10665/349321/WHO-2019-nCoV-clinical-2021.2-eng.pdf

WHO. (2021c). COVID-19 natural immunity. file:///C:/Users/AHCS8330/Downloads/WHO-2019-nCoV-Sci-Brief-Natural-immunity-2021.1-eng.pdf

WHO. (2022a, November 11). Coronavirus disease (COVID-19) Pandemic. World Health Organization. https://www.who.int/emergencies/diseases/novel-coronavirus-2019

WHO. (2022b, 2022). Middle East respiratory syndrome coronavirus (MERS-CoV). World Health Organization. Retrieved 08/19/2020 from https://www.who.int/emergencies/mers-cov/en/

WHO. (2022c). SARS (Severe Acute Respiratory Syndrome). World Health Organization. Retrieved 04/19/2022 from https://www.who.int/ith/diseases/sars/en/

WHO. (2023, March 28). Coronavirus disease (COVID-19). Retrieved 05/20/2020 from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/coronavirus-disease-covid-19

Woof, J. M., & Kerr, M. A. (2006). The function of immunoglobulin A in immunity. The Journal of Pathology, 208(2), 270-282. https://doi.org/10.1002/path.1877

Wu, F., Liu, M., Wang, A., Lu, L., Wang, Q., Gu, C., Chen, J., Wu, Y., Xia, S., Ling, Y., Zhang, Y., Xun, J., Zhang, R., Xie, Y., Jiang, S., Zhu, T., Lu, H., Wen, Y., & Huang, J. (2020). Evaluating the Association of Clinical Characteristics With Neutralizing Antibody Levels in Patients Who Have Recovered From Mild COVID-19 in Shanghai, China. JAMA Intern Med. https://doi.org/10.1001/jamainternmed.2020.4616

Wulff, N. H., Tzatzaris, M., & Young, P. J. (2012). Monte Carlo simulation of the Spearman-Kaerber TCID50. J Clin Bioinforma, 2(1), 5. https://doi.org/10.1186/2043-9113-2-5

Xiao, D. A. T., Gao, D. C., & Zhang, D. S. (2020). Profile of Specific Antibodies to SARS-CoV-2: The First Report. J Infect. https://doi.org/10.1016/j.jinf.2020.03.012

Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H., Wu, Y., Zhang, L., Yu, Z., Fang, M., Yu, T., Wang, Y., Pan, S., Zou, X., Yuan, S., & Shang, Y. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med, 8(5), 475-481. https://doi.org/10.1016/s2213-2600(20)30079-5

Yau, F., Ferreira, R., Kamali, R., Bird, P. W., Halliwell, R., Patel, H., Nicoara, D. C., Woltmann, G., & Tang, J. W. (2021). Clinical utility of a rapid 'on-demand' laboratory-based SARS-CoV-2 diagnostic testing service in an acute hospital setting admitting COVID-19 patients. Clin Infect Pract, 12, 100086. https://doi.org/10.1016/j.clinpr.2021.100086

Yelin, I., Aharony, N., Shaer Tamar, E., Argoetti, A., Messer, E., Berenbaum, D., Shafran, E., Kuzli, A., Gandali, N., Shkedi, O., Hashimshony, T., Mandel-Gutfreund, Y., Halberthal, M., Geffen, Y., Szwarcwort-Cohen, M., & Kishony, R. (2020). Evaluation of COVID-19 RT-qPCR test in multi-sample pools. Clin Infect Dis. https://doi.org/10.1093/cid/ciaa531

Zhang, Y. V., Wiencek, J., Meng, Q. H., Theel, E. S., Babic, N., Sepiashvili, L., Pecora, N. D., Slev, P., Cameron, A., Konforte, D., & the, A. C. S. T. T. F. (2021). AACC Practical Recommendations for Implementing and Interpreting SARS-CoV-2 EUA and LDT Serologic Testing in Clinical Laboratories. Clinical Chemistry. https://doi.org/10.1093/clinchem/hvab051

Zhao, J., Yuan, Q., Wang, H., Liu, W., Liao, X., Su, Y., Wang, X., Yuan, J., Li, T., Li, J., Qian, S., Hong, C., Wang, F., Liu, Y., Wang, Z., He, Q., Li, Z., He, B., Zhang, T., . . . Zhang, Z. (2020). Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciaa344 Medical Director Review 7/2022

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Policy Implementation/Update Information

9/13/22	New policy developed. BCBSNC will provide coverage for Coronavirus Testing in the Outpatient Setting when medical criteria and guidelines outlined in the policy are met. Medical Director review 7/2022. **Notification give 9/13/2022 for effective date 11/15/2022.** (tt)
12/13/22	Policy title updated to include "AHS-G2174" to align with Avalon. (tt)
2/7/23	Reviewed by Avalon 4th Quarter 2022 CAB. Medical Director Review 12/2022. Description, Policy Guidelines, and References updates. Related polices section removed. Update When Not Covered #4 to read as follows: "Reimbursement is not allowed for host antibody serology testing for all other situations not described above." Remaining coverage criteria updated for clarity. (tt)

8/15/23 Reviewed by Avalon 2nd Quarter 2023 CAB. Added related policies. Added the following statement to Benefits Application: "This policy only addresses testing for the purpose of medical decision making in the outpatient setting. This policy does not address work, school, state, or federally mandated SARS-CoV-2 testing". Removed the following from when covered section: "For asymptomatic individuals prior to undergoing immunosuppressive or aerosol-producing procedures." As new guidelines specify that asymptomatic screening for those without a known exposure is not supported, even if they are going to undergo these types of procedures. Added "Reimbursement is not allowed for SARS-CoV-2 genotyping in the outpatient setting." to not covered section. Remaining coverage criteria updated for clarity and consistency. Added CPT code 87913 and removed CPT codes 87797, 87799, G2023, G2024, U0003, U0004 and U0005. Medical Director Review 7/2023. (tt)

9/29/23 Added CPT code 0408U to Billing/Coding section, effective 10/1/2023. (tt)