Coronavirus Testing in the Outpatient Setting

Policy Number: AHS – G2174 – Coronavirus Testing in the Outpatient Setting	Prior Policy Name and Number, as applicable:
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I. Policy Description

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, 2020d). As of January 28, 2021, the United States had reported more than 25,651,968 confirmed cases of COVID-19 and over 430,643 reported COVID-19 deaths (JHU, 2021). 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.

II. Related Policies

Policy Number	Policy Title
AHS-G2060	Fecal Analysis in the Diagnosis of Intestinal Dysbiosis
AHS-G2149	Pathogen Panel Testing
AHS-M2097	Identification of Microorganisms Using Nucleic Acid Probes

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies

and coverage, please visit their search website http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx?from2=search1.asp& or the manual website

1. Targeted nucleic acid testing, such as RT-PCR, for COVID-19 (SARS-CoV-2) including rapid molecular tests **MEETS COVERAGE CRITERIA** in the following situations:
    a. Individuals displaying signs and symptoms of possible COVID-19 infection (See Note 1).
    b. Asymptomatic individuals with known exposure to COVID-19
    c. Asymptomatic individuals prior to undergoing immunosuppressive or aerosol-producing procedures
2. Targeted nucleic acid testing, such as RT-PCR, **MEETS COVERAGE CRITERIA** for detection of severe acute respiratory syndrome (SARS) coronavirus RNA in persons with signs or symptoms of SARS who have traveled to endemic areas or have been exposed to persons with SARS.
3. Targeted nucleic acid testing, such as RT-PCR, **MEETS COVERAGE CRITERIA** for detection of Middle East respiratory syndrome (MERS) coronavirus RNA in persons with signs or symptoms of MERS who have traveled to endemic areas or have been exposed to persons with MERS.
4. Host antibody serology testing to support a diagnosis of Multisystem Inflammatory Syndrome in Children (MIS-C) or Multisystem Inflammatory Syndrome in Adults (MIS-A) **MEETS COVERAGE CRITERIA** (See Notes 2 and 3).
5. The use of an antigen-detecting diagnostic test in symptomatic individuals, including antigen rapid tests, for SARS-CoV-2, **MEETS COVERAGE CRITERIA**.
6. Multiplex PCR-based panel testing of up to **5** respiratory pathogens **MEETS COVERAGE CRITERIA** for patients with signs and symptoms of a respiratory tract infection, as evidenced by a compatible clinical syndrome including at least one of the following: temperature of 102 or greater, pronounced dyspnea, tachypnea, or tachycardia.
7. Antigen panel testing of up to 5 antigens **MEETS COVERAGE CRITERIA** for patients with signs and symptoms of a respiratory tract infection, as evidenced by a compatible clinical syndrome including at least one of the following: temperature of 102 or greater, pronounced dyspnea, tachypnea, or tachycardia.
8. Antigen panel testing of **6 or more** antigens **DOES NOT MEET COVERAGE CRITERIA**.
9. Multiplex PCR-based panel testing of **6 or more** respiratory pathogens **DOES NOT MEET COVERAGE CRITERIA**.

10. Host antibody serology testing to diagnose an acute suspected COVID-19 (SARS-CoV-2) infection or any other human coronavirus infection **DOES NOT MEET COVERAGE CRITERIA** (except for MIS-C testing in children or MIS-A testing in adults).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

11. Host antibody serology testing to determine immunity status for any human coronavirus **DOES NOT MEET COVERAGE CRITERIA**.

12. Neutralization antibody testing for SARS-CoV-2 **DOES NOT MEET COVERAGE CRITERIA** for any indications**.**

13. Testing for other endemic coronaviruses, such as 229E, NL63, OC43, and HKU1, **DOES NOT MEET COVERAGE CRITERIA**.

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, 2020n; WHO, 2020j).

Note 2: According to the CDC, evidence of possible MIS-C includes (CDC, 2020h):

- 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, 2020i)"
- 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):

- 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).

IV. Reimbursement

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

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

V. Scientific Background

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, 2020f). 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, 2020k). 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, the WHO reports 2562 laboratory-confirmed cases of MERS with 881 MERS-associated deaths (34.4% fatality rate) in 27 countries (WHO, 2020h).

Unlike the initial SARS and MERS outbreaks that were predominantly regionally contained, COVID-19 has been a global pandemic. According to the WHO, as of January 28,2021, there were more than 100 million confirmed cases of COVID-19 in 223 countries with over 2,166,440 confirmed deaths (WHO, 2020d). 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, 2020b). 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, 2020). Sepsis, multiorgan failure (including the kidney, liver, and heart), pneumonia, and acute respiratory distress syndrome (ARDS) can also occur (WHO, 2020c; 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, 2020).

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, 2020i; DeBiasi et al., 2020; Jones et al., 2020; Verdoni et al., 2020; WHO, 2020i). 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).

On September 20, 2020, the first case of a new COVID-19 variant, B.1.1.7 or VUI-202012/01 (VUI for "variant under investigation") was recorded in the UK. It is defined by 17 mutations, one of the most significant being a N501Y mutation in the virus spike protein that binds to the human ACE2 receptor. As of December 13, 2020, 1108 cases were identified in the UK; since then, it has spread worldwide to the US and Canada, so incidence has certainly increased (CDC, 2021c; Wise, 2020). Primarily, this variant was concentrated in the southeast and east of England, as well as south Wales and Cumbria. It is defined by a higher rate of transmission (71% higher than for other variants), and may have a higher viral load, according to the UK's New and Emerging Respiratory Virus Threats Advisory Group (Mahase, 2020). As a result of the new variant's increased infectiousness, the UK reported more than 50,000 cases a day at the end of December 2020 and beginning of January 2021 (Kirby, 2021). Children

may be more susceptible to this COVID-19 variant (Mahase, 2020). However, there is currently no evidence that the B.1.1.7 variant has an increased severity of illness or risk of death (CDC, 2021c; Tang, Tambyah, & Hui, 2020). As of January 27, 2021, there are currently 315 total B.1.1.7 lineage cases in the US (CDC, 2021h). Other variants, including those discovered in South Africa (B.1.351 variant) and Brazil (P.1), have circulated worldwide, but do not have as many mutations or any cases in the US (CDC, 2021c).

As of January 15, 2021, the CDC has listed the Pfizer-BioNTech COVID-19 Vaccine and Moderna COVID-19 Vaccine as the only two authorized and recommended vaccines to prevent COVID-19 in the US (CDC, 2021a). Both types 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. They both require two doses to obtain optimal effectiveness (CDC, 2021g). After the two doses, the Pfizer vaccine and Moderna vaccine are 95% and 94.1% effective, respectively, in preventing COVID-19 (Branswell, 2020). Three vaccines remain in Phase 3 clinical trials as of December 28, 2020 in the US; these include vaccines from AstraZeneca, Janssen, and Novavax (CDC, 2021a), but AstraZeneca is already approved for usage in the EU (AstraZeneca, 2021). AstraZeneca and Janssen are vector vaccines, which contain weakened versions of a virus that has the same entryway as COVID-19 into a cell, and Novavax is a protein subunit vaccine, which contains stabilized but immunogenic pieces of the SARS-CoV-2 virus, namely the spike protein (AstraZeneca, 2021; CDC, 2021g; NIH, 2020b). AstraZeneca and Novavax COVID-19 vaccines require two doses, whereas Janssen's COVID-19 vaccine only requires one dose (AstraZeneca, 2021; Roberts, 2021). However, the Janssen vaccine is only 66% effective, and the Astrazeneca and Novavax vaccines have been found to be up to 90% effective in UK trials (Roberts, 2021; Van Beusekom, 2020).

Vaccines in clinical trials have focused on targeting the spike (S) protein (based on experience with SARS-CoV-1), which contains a receptor binding domain (RBD) responsible for allowing entrance into host cells, and is the current target for neutralizing antibodies. Other targets could potentially include the nucleocapsid (N) protein, likely for inactivated virus or live attenuated approaches, or T cell epitopes, which may provide additional protection. Knowing that T-cell responses against structural proteins in SARS-CoV-1 were more immunogenic than non-structural proteins could be key in manufacturing more effective vaccines for SARS-CoV-2 as well (Tregoning et al., 2020).

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, Lienau, & Witzenrath, 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, 2020l). 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 (CT imaging) to supplement isolation, discharge, and any transfers during this epidemic (Li et al., 2020).

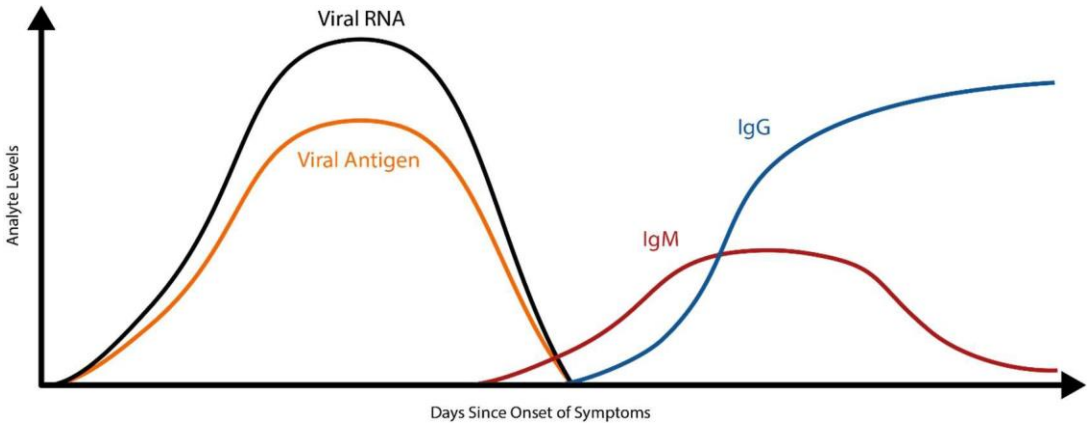

Figure 1: *General time course of a viral infection, such as SARS-CoV-2. This is for illustrative purposes and should not be used as a primary reference or for diagnostic purposes. The original content can be found within the references (The_Native_Antigen_Company, 2020).*

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, Reucher, Nörz, & Lütgehetmann, 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, Simundic, & Plebani, 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, 2020k). 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, Klinkenberg, & Wallinga, 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).

	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)

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

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).

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, Skvaril, Noseda, & Barandun, 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, Cosma, Sciacovelli, Faggian, & Plebani, 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, 2020g; 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, 2020g).

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 May 11, 2020, they state, "Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 coronavirus infection (FDA, 2020g)." The FDA also acknowledges that antibody testing limitations include the following:

- *A positive result does not distinguish between a past and current infection.*
- *A positive result may be due to cross-reactivity with immunoglobulins from an infection due to a different virus, such as coronavirus HKU1, NL63, OC43, or 229E.*
- *A negative result does not preclude acute SARS-CoV-2 infection.*

The FDA published a Frequently Asked Questions (FAQ) update on July 29, 2020. 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, 2020b).

Since SARS-CoV-2 is a new, emerging virus with little research to date, 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, Gao, & Zhang, 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, Braliou, Dimou, Nikolopoulos, and Bagos (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).

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, 2020b). This test has been approved as a point-of-care (POC) test (FDA, 2020e). 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, 2020b). 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 January 30, 2021, thirteen antigen tests have Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA) (FDA, 2021b). Eight of these tests are available as rapid, point-of-care testing platforms including: Sofia®2 SARS Antigen FIA lateral flow immunofluorescent sandwich assay (Quidel_Corporation, 2020b) approved May 8, 2020, BD Veritor System for Rapid Detection of SARS-CoV-2 (BD_Veritor, 2020) approved July 2, 2020, LumiraDx SARS-CoV-2 Ag Test (LumiraDx, 2020) approved August 18, 2020, BinaxNOW COVID-19 Ag Card (Abbott_Diagnostics, 2020) approved August 26, 2020, Sofia 2 Flu + SARS Antigen FIA (Quidel_Corporation, 2020a) approved October 2, 2020, *CareStart* COVID-19 Antigen test (Access_Bio, 2020) approved October 13, 2020, Clip COVID Rapid Antigen Test approved December 7, 2020, and QuickVue SARS Antigen Test approved December 18, 2020. The Ellume COVID-19 Home Test, approved December 15, 2020, and BinaxNOW COVID-19 antigen card home test, approved December 16, 2020, are the only two home

tests that were issued an EUA. Sampinute COVID-19 Antigen MIA (Celltrion_USA, 2020) approved on October 23, 2020, Simoa SARS-CoV-2 N Protein Antigen Test approved January 5, 2021, and VITROS immunodiagnostic products SARS-CoV-2 antigen reagent pack approved January 11, 2021, are not currently for use in patient care settings.

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, Tzatzaris, & Young, 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 include 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, 2020b)

- 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, 2020b)."
- 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 05/18/2020. 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, 2020a).

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).

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 November 6, 2020, the BioFire® Respiratory Panel 2.1 (RP2.1), the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, ePlex Respiratory Pathogen Panel 2, and the Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay from the CDC had received an EUA from the FDA for testing for COVID-19 (FDA, 2020e). 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, 2020e).

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	• Adenovirus • HCoV 229E • HCoV HKU1 • HCoVNL63 • HCoV OC43 • SARS-CoV-2 • Human Metapneumovirus A+B • Influenza A	• Adenovirus • HCoV 229E • HCoV HKU1 • HCoVNL63 • HCoV OC43 • SARS-CoV-2 • Human Metapneumovirus A+B • Influenza A

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*
	○ 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*
		○ 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 (WRCEVA), 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 positive 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, 2020c).

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).

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" (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" (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" (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, 2020). The FDA 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).

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, Parmar, Anand, Srikanth, & Saritha, 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, 2020d) 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, Sahoo, and Pinsky (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).

VI. Guidelines and Recommendations

World Health Organization (WHO, 2020a, 2020e, 2020g, 2020i)

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, 2020e). Firstly, 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, 2020e).

The WHO released a scientific brief with recommendations for the use of SARS-CoV-2 Ag-RDTs. 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 infection in a range of settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility" (WHO, 2020a). Ag-RDTs should be conducted within 5-7 days after the onset of symptoms. WHO does not recommend using SARS-CoV-2 Ag-RDTs when:

- The individual is asymptomatic (unless the person is a contact of a confirmed case)
- The area has zero or sporadic cases, as Ag-RDTs are not recommended for routine surveillance
- The area does not have appropriate personal protective equipment (PPE)
- For airport or border screening at points of entry
- Management of patient does not change based on results of the test
- In screening prior to blood donation

Overall, they suggest that "Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19." However, "there is insufficient evidence on performance and operational use to recommend specific commercial products" (WHO, 2020a)

WHO released a second scientific brief with recommendations concerning immunity passports (WHO, 2020g) 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, 2020g)".

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 ECHO findings or elevated Troponin/NT-proBNP),
    - Evidence of coagulopathy (by PT, PTT, elevated d-Dimers).
    - Acute gastrointestinal problems (diarrhoea, 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, 2020i)."

Centers for Disease Control and Prevention (CDC, 2020b, 2020f, 2020g, 2020j, 2020k, 2020m, 2021b, 2021d; CDC & OSHA, 2020a, 2020b)

In the update of the CDC guidelines *Overview of Testing for SARS-CoV-2,* dated October 21, 2020, the CDC states that "viral [nucleic acid or antigen] tests are recommended to diagnose acute infection" (CDC, 2020k) Regarding use of antibody testing and acute infection, the CDC remarks: "CDC does not currently recommend using antibody testing as the sole basis for diagnosis of acute infection, and antibody tests are not authorized by FDA for such diagnostic purposes. In certain situations, serologic assays may be used to support clinical assessment of persons who present late in their illnesses when used in conjunction with viral detection tests. In addition, if a person is suspected to have post-infectious syndrome (e.g., Multisystem Inflammatory Syndrome in Children) caused by SARS-CoV-2 infection, serologic assays may be used" (CDC, 2020k).

The guideline also describes five populations for which SARS-CoV-2 testing with viral tests (i.e., nucleic acid or antigen tests) is "appropriate", which are listed below (CDC, 2020m):

- "Individuals with signs or symptoms consistent with COVID-19"
- "Asymptomatic individuals with recent known or suspected exposure to SARS-CoV-2 to control transmission"
- "Asymptomatic individuals without known or suspected exposure to SARS-CoV-2 for early

identification in special settings"

- "Resolution of infection (e.g. discontinuation of home isolation)"
- "Public health surveillance for SARS-CoV-2"

An additional update from January 21, 2021, regarding testing for a current infection, reaffirmed the necessity of testing the above cohorts for COVID-19. This guideline recommended that the following groups receive testing: "people who have symptoms of COVID-19, people who have had close contact (within 6 feet of an infected person for a cumulative total of 15 minutes or more over a 24-hour period) with someone with confirmed COVID-19, people who have taken part in activities that put them at higher risk for COVID-19 because they cannot socially distance as needed, such as travel, attending large social or mass gatherings, or being in crowded indoor settings, and people who have been asked or referred to get testing by their healthcare provider, local, external, or state health department (CDC, 2021e).

The CDC recommends using "authorized nucleic acid or antigen detection assays that have received an FDA EUA to test persons with symptoms when there is a concern of potential COVID-19." 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. The CDC also notes the possible use of "expanded testing", such as testing of every individual in a shared setting, such as an office. The CDC defers to "local, territorial, tribal, and state health departments", stating that individual facilities should have a plan of possible actions based on potential test results. In general, the CDC remarks that viral testing is "diagnostic" when conducted among symptomatic patients, asymptomatic patients with known or suspected exposure to SARS-CoV-2, and to determine infection resolution. Viral testing is considered "screening" when performed on asymptomatic patients without known exposure to SARS-CoV-2.

Finally, the update included new comments on testing for resolution of infection, with the CDC now stating "Except for rare situations, a test-based strategy is no longer recommended to determine when an individual with SARS-CoV-2 infection is no longer infectious (e.g., to discontinue Transmission-Based Precautions or home isolation)." The CDC observes that the test-based strategy resulted in prolonged isolation of patients who had detectable SARS-CoV-2 RNA but were no longer infectious. Certain situations, such as severely immunocompromised patients may benefit from the test-based strategy as they may be infectious for an extended time, but for all other circumstances, the CDC recommends "the symptom-based strategy should be used to determine when to discontinue Transmission-Based Precautions or when HCP can return to work" (CDC, 2020m).

The CDC also published *Interim Guidelines for COVID-19 Antibody Testing* on August 1, 2020. The CDC states that "Currently, there is no identified advantage whether the assays test for IgG, IgM and IgG, or total antibody." The CDC also acknowledges the potential application of neutralizing antibody detection (as opposed to binding antibody detection), but remarks that the FDA has not authorized any neutralization tests for SARS-CoV-2 at time of publication (CDC, 2020g).

Regarding testing for past infections, the CDC recommends that antibody tests should not be used to diagnose a current COVID-19 infection, except when viral testing is delayed (CDC, 2021f)

Within the CDC's *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)*, they recommend collecting and testing upper respiratory samples for initial diagnostic testing for SARS-CoV-2. Within their recommendation, they list the following (without stating a preference) as acceptable specimens:

- "A nasopharyngeal (NP) specimen collected by a trained healthcare provider
- An oropharyngeal (OP) specimen collected by a trained healthcare provider
- A nasal mid-turbinate swab collected by a trained healthcare provider or by a supervised or unsupervised onsite self-collection (using a flocked tapered swab), or self-collected at home following kit collection instructions
- An anterior nares specimen collected by a trained healthcare provider or by a supervised or unsupervised onsite self-collection or self-collected at home following kit instructions (using a flocked or spun polyester swab)
- Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimen collected by a trained healthcare provider
- A saliva specimen collected by the person being tested, either at home or at a testing site under supervision" (CDC, 2021d).

The CDC issued employer-based guidelines titled *Guidance for Businesses and Employers: Plan, Prepare, and Respond to Coronavirus Disease 2019*. Within the guidelines, they recommend that symptomatic employees should notify their supervisor and stay home. "Employees who are sick with COVID-19 should isolate and follow CDC-recommended steps. Employees who are asymptomatic (have no symptoms) or pre-symptomatic (not yet showing symptoms) but have tested positive for SARS-CoV-2 infection should also isolate and follow CDC-recommended steps. Employees should not return to work until the criteria to discontinue home isolation are met, in consultation with healthcare providers. Employees who are well but who have a sick household member with COVID-19 should notify their supervisor and follow CDC-recommended precautions" (CDC, 2021b).

The CDC goes on to state, "Employers should not require a COVID-19 test result or a healthcare provider's note for employees who are sick to validate their illness, qualify for sick leave, or to return to work. Under the Americans with Disabilities Act, employers are permitted to require a doctor's note from their employees to verify that they are healthy and able to return to work. However, as a practical matter, be aware that healthcare provider offices and medical facilities may be extremely busy and not able to provide such documentation in a timely manner. Review human resources policies to make sure that policies and practices are consistent with public health recommendations and are consistent with existing state and federal workplace laws" (CDC, 2021b).

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, 2020c). Within these guidelines, they recommend possible screening and monitoring methods for employers to use due to recent outbreaks within this industry. These include verbal screening, temperatures, and engineering controls (such as physical barriers/dividers), but the guidelines are silent on laboratory testing. Within the section addressing when a previously ill (or asymptomatic) employee can return to work, they recommend that the employers should follow the interim guidelines of the CDC titled *Discontinuation of Isolation for Persons with COVID-19 Not in Healthcare Settings* (CDC & OSHA, 2020c).

Likewise, the CDC-OSHA joint interim guidelines for Manufacturing Workers and Employers state that "[w]orkplaces, particularly in areas where community transmission of COVID-19 is occurring, should consider developing and implementing a comprehensive screening and monitoring strategy aimed at preventing the introduction of COVID-19 into the work site. Consider a program of screening workers

before entry into the workplace, criteria for exclusion of sick workers, including asymptomatic workers who have tested positive for COVID-19; and criteria for return to work of exposed and recovered (those who have had signs or symptoms of COVID-19 but have gotten better." Concerning the reintegration of exposed, asymptomatic individuals, they recommend following the CDC's guide titled *CDC Critical Infrastructure Guidance*. For the reintegration of individuals "with COVID-19 (COVID-19 positive), including those workers who have remained asymptomatic", they recommend following the CDC interim guidance, *Discontinuation of Isolation for Persons with COVID-19 Not in Healthcare Settings* (CDC & OSHA, 2020a).

As of December 3, 2020, the CDC no longer recommends using a test-based strategy to decide whether to continue home isolation, except in certain circumstances. The CDC now recommends a symptom-based strategy. The testing-relevant recommendations are listed below:

- "**Persons infected with SARS-CoV-2 who never develop COVID-19 symptoms** may discontinue isolation and other precautions 10 days after the date of their first positive RT-PCR test for SARS-CoV-2 RNA."
- "RT-PCR testing for detection of SARS-CoV-2 RNA for discontinuing isolation could be considered for persons who are severely immunocompromised, in consultation with infectious disease experts. For all others, a test-based strategy is no longer recommended except to discontinue isolation or other precautions earlier than would occur under the symptom-based strategy outlined above."

The CDC writes that the test-based strategy will still require "negative results using RT-PCR for detection of SARS-CoV-2 RNA under an FDA Emergency Use Authorization (EUA) for COVID-19 from at least two consecutive respiratory specimens collected ≥24 hours apart (total of two negative specimens)."

The symptom-based strategy is as follows:

"Persons with COVID-19 who have symptoms and were directed to care for themselves at home may discontinue isolation under the following conditions:

- "At least 10 days have passed since symptom onset and
- At least 24 hours have passed since resolution of fever without the use of fever-reducing medications and
- Other symptoms have improved.

With regards to the 10 days past symptom onset, the CDC notes, "a limited number of persons with severe illness may produce replication-competent virus beyond 10 days, that may warrant extending duration of isolation for up to 20 days after symptom onset."

The CDC includes a footnote to the guidelines stating, "All test results should be final before isolation is ended. Testing guidance is based upon limited information and is subject to change as more information becomes available. In persons with a persistent productive cough, SARS-CoV-2-RNA might be detected for longer periods in sputum specimens than in respiratory specimens" (CDC, 2020b).

The CDC notes that the 10 days interval described above primarily refers to mild to moderate cases, whereas more severe cases may be infectious for up to 20 days after symptom onset. However, the CDC has noted recovered patients may shed detectable SARS-CoV-2 RNA for up to 3 months after symptom onset. The CDC writes that re-testing asymptomatic patients in this period is "unlikely to

yield useful information, even if the person had close contact with an infected person". The CDC includes some recommendations regarding the role of PCR testing after "discontinuation of isolation or precautions":

- "For persons previously diagnosed with symptomatic COVID-19 who remain asymptomatic after recovery, retesting is not recommended within 3 months after the date of symptom onset for the initial COVID-19 infection."
- "For persons who develop new symptoms consistent with COVID-19 during the 3 months after the date of initial symptom onset, if an alternative etiology cannot be identified by a provider, then the person may warrant retesting. Consultation with infectious disease or infection control experts is recommended, especially in the event symptoms develop within 14 days after close contact with an infected person. Persons being evaluated for reinfection with SARS-CoV-2 should be isolated under recommended precautions while undergoing evaluation. If reinfection is confirmed or remains suspected they should remain under the recommended SARS-CoV-2 isolation until they meet the criteria for discontinuation of precautions – for most persons, this would be 10 days after symptom onset and resolution of fever for at least 24 hours, without the use of fever-reducing medications, and with improvement of other symptoms."
- "For persons who never developed symptoms, the date of first positive viral diagnostic test (PCR or antigen) for SARS-CoV-2 RNA should be used in place of the date of symptom onset."

Finally, the CDC does not recommend serologic testing to "establish the presence or absence of SARS-CoV-2 infection or reinfection" (CDC, 2020c).

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).

Centers for Medicare & Medicaid Services (Wright & CMS, 2020)

The Centers for Medicare & Medicaid Services (CMS) released recommendations regarding the reopening of nursing homes for state and local officials. Concerning testing, CMS recommends that each facility should have a plan that at a minimum consider the following:

- The capacity to test all nursing home residents once with a single baseline COVID-19 test.
- Similarly, the capacity to retest all residents upon identification of a resident with symptoms consistent of COVID-19 or if a staff member tests positive for the disease. Each resident should be re-tested weekly until every resident tests negative.
- The capacity for all staff members, including all volunteers and vendors who are on site on a weekly basis, to receive a single baseline COVID-19 test and to have weekly re-testing of all staff. CMS notes that state and local governments may adjust the frequency of testing based on the circulation of the virus in the community.
- Written screening protocols should be available for all staff, all residents, and all persons entering the facility, including visitors.
- The tests used to screen for COVID-19 should have greater than 95% sensitivity and greater than 90% specificity with the results obtained rapidly (within 48 hours).
- Antibody testing should not be used for diagnosis of an active COVID-19 infection.
- Any staff or resident who either refuses or is unable to comply is to be considered positive (Wright & CMS, 2020).

In a "Frequently Asked Questions" publication from June 19, 2020, the CMS writes that the potential risks of using pooled samples include "a risk of obtaining false negative or false positive results when utilizing a pooled sampling testing model" and that "all positive and inconclusive SARS-CoV-2 results from pooled sampling must be confirmed by having each participant whose sample was contained within the cohort to be tested by a CLIA-certified facility." The CMS notes that they do not have oversight authority over facilities that are not CLIA-certified (CMS, 2020).

National Institutes of Health (NIH, 2020a, 2020c)

The NIH released COVID-19 treatment guidelines, and within the 12/17/2020 update the addressed laboratory in asymptomatic and presymptomatic individuals. They give a level AIII (Strong

recommendation; Expert Opinion) recommendation stating, "Some asymptomatic individuals have been reported to have objective radiographic findings that are consistent with COVID-19 pneumonia. The availability of widespread virologic testing for SARS-CoV-2 and the development of reliable serologic assays for antibodies to the virus will help determine the true prevalence of asymptomatic and presymptomatic infection" (NIH, 2020a).

The NIH also released COVID-19 testing guidelines (updated 12/17/2020). Their COVID-19 Treatment Guidelines Panel recommends that a nucleic acid amplification test (NAAT) for SARS-CoV-2 should be used to diagnose an acute SARS-CoV-2 infection (Level AIII). Regarding antigen-based diagnostic tests, the NIH states that they perform best early in the course of symptomatic infection, when the viral load is highest. "When a person who is strongly suspected of having SARS-CoV-2 infection receives a negative result on an initial antigen test, repeat testing using a NAAT should be considered." However, the panel recommends against using serologic testing to determine immunity to SARS-CoV-2 infection (level AIII). The Panel also recommends against serologic testing as the "sole basis" for diagnosis of an acute SARS-CoV-2 infection (level AIII) (NIH, 2020c).

American Medical Association (AMA, 2020)

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 (AMA, 2020):

- "Use of serology tests should currently be limited to population-level seroprevalance 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."

  "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, 2020b, 2020d; Miller et al., 2018)

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, 2020c). 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, 2020c):

- 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

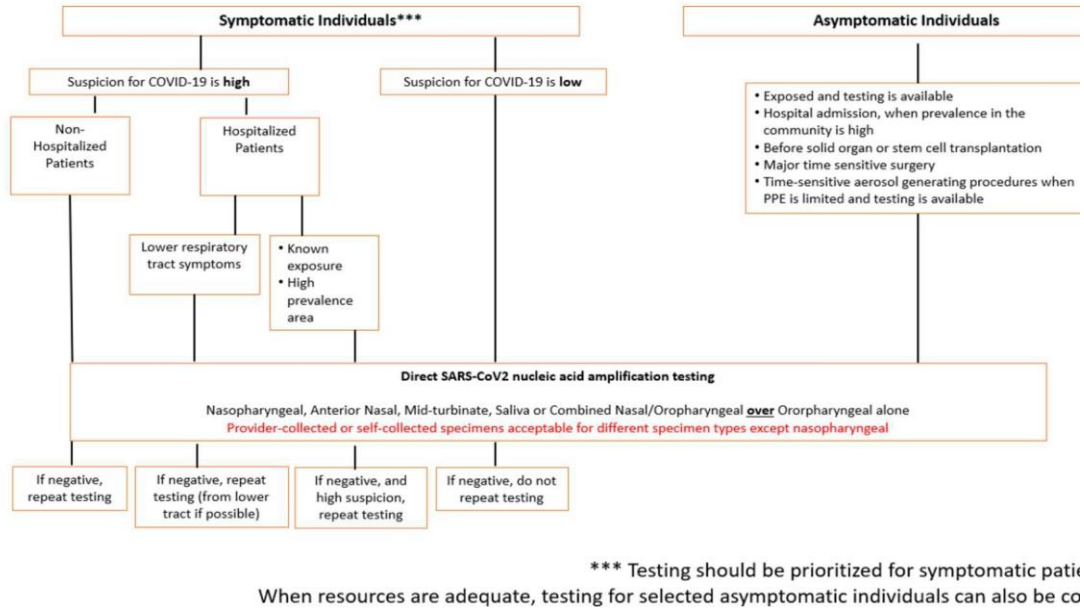

Figure 2: *IDSA Algorithm for SARS-CoV-2 Nucleic Acid Testing* (IDSA, 2020a). The Infectious Diseases Society of America (IDSA) released their algorithm for nucleic acid testing for COVID-19. According to the IDSA guidelines, testing priority should first be given to symptomatic patients; if resources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

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, 2020d).

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).

Occupational Safety and Health Administration (OSHA, 2020)

In the OSHA guidelines for employers, they classify jobs based on exposure risk to COVID-19 (lower, medium, high, or very high). In general, they recommend following CDC and local health department guidelines. They do state that employers should not require documentation for employees to return to work since healthcare provider offices and medical facilities may be extremely busy during the crisis. For jobs classified at medium exposure risk, administrative controls do include the responsibility of the employer to "communicate the availability of medical screening or other worker health resources (e.g., on-site nurse; telemedicine services)." For jobs classified at high or very high exposure risk, administrative controls include that employers should "consider offering enhanced medical monitoring of workers during COVID-19 outbreaks" (OSHA, 2020). OSHA does not state what the term enhanced medical monitoring entails.

European Centre for Disease Prevention and Control (ECDC, 2020a, 2020b, 2020c)

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. They also recommend a positive patient end either isolation at home (or comparable safe place) or hospitalization after clinical improvement and two negative RT-PCR test results (from a respiratory sample) taken at least 24 hours apart at least eight days after the original onset of symptoms (ECDC, 2020b).

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, 2020a):

- "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 turnaround time should be minimised, 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 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 immediately 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 sub-national areas that have achieved sustained control of SARSCoV-2 circulation 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."

Finally, the ECDC notes that "antibodies against SARS-CoV-2 become detectable typically 10–14 days after infection, meaning that antibody tests are only useful to confirm a prior infection, and a positive result does not imply protective immunity against SARS-CoV-2 reinfection" (ECDC, 2020a).

Public Health England (PHE, 2020)

The PHE does not recommend the use of rapid tests for the diagnosis of COVID-19 infection in community settings, such as pharmacies, because "there is little information on the accuracy of these rapid point of care tests, or on how a patient's antibody response develops or changes during COVID-19 infection. It is not known whether either a positive or negative result is reliable" (PHE, 2020).

American Academy of Pediatrics (AAP, 2020a, 2020b)

The AAP published the following guideline to "help pediatric practices determine when to test for severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection in their patient population". The AAP writes that the most common scenarios for testing include symptomatic patients, patients

with exposure to a person with COVID-19 infection, and patients scheduled for elective surgery. The AAP recommends that a child receive testing 24-72 hours before their surgery.

The AAP notes that molecular testing, particularly a PCR test with FDA approval, is the "gold" standard, and that in general, antigen (rapid) tests "are best used on patients with symptoms who are in settings where timing is important to notify others in efforts to contain disease (e.g., in-person school, daycare, etc) and where a follow-up PCR test can be performed if needed. For purposes of clearance for a specific setting such as return to school, however, a negative antigen test result will often not suffice. The use of antigen tests in asymptomatic patients is generally not recommended because of the very low pretest probability and risk for false positivity." Further, the AAP states that antibody 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 confirm that a patient has protection against SARS-CoV-2. 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, 2020b).

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 CDC defines an MIS-C case by the following criteria:

- "An individual aged <21 years presenting with:
  - fever (24 hours or more),
  - laboratory evidence of inflammation (CRP, ESR, fibrinogen; procalcitonin, d-dimer, ferritin, LDH, or IL-6, neutrophilia, lymphopenia, low albumin),
  - evidence of clinically severe illness requiring hospitalization,
  - with multisystem (>2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND
- No alternative plausible diagnoses; AND
- Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms.

The CDC delineates a testing algorithm for MIS-C as follows:

- "A child who presents with a persistent fever (>3 days) and who is moderately to severely ill with clinical signs of organ dysfunction should be evaluated."

- "Initial laboratory screening may include a complete blood cell count (CBC) with differential, urine analysis, ESR, CRP, ferritin, LDH, comprehensive metabolic panel, pro-BNP, troponin, and fibrinogen. Laboratory screening for systemic inflammation may also be considered."

- "Hospitalized children may need an expanded laboratory and cardiac workup.

- COVID-19 testing should be performed with RT-PCR assay and serologic testing in all cases. If all tests are initially negative, serology may be needed and must be sent prior to administration of Intravenous Immunoglobulin (IVIG).
- Cardiac workup may include a chest radiograph, echocardiogram, EKG.
- Expanded laboratory tests (if not already conducted) may include troponin, 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" (AAP, 2020a).

American College of Rheumatology (Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, MB, et al., 2020)

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).

VII. 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, 2020e). 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, 2020f)." As of January 29, 2021, the FDA had issued 320 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).

Additionally, 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). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

Billing applicable codes is not a guarantee of payment; see Section III for indications and limitations of coverage that may affect payment

CPT	Code Description
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (eg, reagent strip)
86328	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (eg, reagent strip); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
86408	Neutralizing antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID19]); screen
86409	Neutralizing antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID19]); titer
86413	Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) (Coronavirus disease [COVID-19]) antibody, quantitative
86769	Antibody; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
86790	Antibody; virus, not elsewhere specified
87426	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; severe acute respiratory syndrome coronavirus (eg, SARS-CoV, SARS-CoV-2 [COVID-19])
87428	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; severe acute respiratory syndrome coronavirus (eg, SARS-CoV, SARS-CoV-2 [COVID-19]) and influenza virus types A and B
87631	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87632	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets
87633	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets

87635	Infectious agent detection by nucleic acid (DNA or RNA);severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism

87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87811	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
0115U	Respiratory infectious agent detection by nucleic acid (DNA and RNA), 18 viral types and subtypes and 2 bacterial targets, amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected Proprietary test: ePlex Respiratory Pathogen (RP) Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0202U	Infectious disease (bacterial or viral respiratory tract infection), pathogen specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected Proprietary test: BioFire® Respiratory Panel 2.1 (RP2.1) Lab/Manufacturer: BioFire® Diagnostics, LLC
0223U	Infectious disease (bacterial or viral respiratory tract infection), pathogen-specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected Proprietary test: QIAstat-Dx Respiratory SARS CoV-2 Panel Lab/Manufacturer: QIAGEN Sciences
0224U	Antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), includes titer(s), when performed Proprietary test: COVID-19 Antibody Test Lab/Manufacturer: Mt Sinai, Mount Sinai Laboratory
0225U	Infectious disease (bacterial or viral respiratory tract infection) pathogen-specific DNA and RNA, 21 targets, including severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected Proprietary test: ePlex® Respiratory Pathogen Panel 2 Lab/Manufacturer: GenMark Dx/GenMark Diagnostics, Inc

0226U	Surrogate viral neutralization test (sVNT), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), ELISA, plasma, serum Proprietary test: Tru-Immune™ Lab/Manufacturer: Ethos Laboratories/GenScript® USA Inc
C9803	Hospital outpatient clinic visit specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19]), any specimen source

G2023	Specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19]), any specimen source
G2024	Specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19]) from an individual in a SNF or by a laboratory on behalf of a HHA, any specimen source
U0001	CDC Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel
U0002	Non-CDC laboratory test for 2019-nCoV (COVID-19), any method
U0003	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique, making use of high throughput technologies as described by CMS-2020-01-R
U0004	2019-nCoV Coronavirus, SARS-CoV-2/2019-nCoV (COVID-19), any technique, multiple types or subtypes (includes all targets), non-CDC, making use of high throughput technologies as described by CMS-2020-01-R
U0005	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique, CDC or non-CDC, making use of high throughput technologies, completed within two calendar days from date and time of specimen collection. (List separately in addition to either HCPCS code U0003 or U0004)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

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X. Revision History

Revision Date	Summary of Changes
06-01-2021	Initial presentation