Evidence-Based Medicine InfoSheet: COVID-19 Diagnostics and Testing

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WHO TO TEST:

The CDC recommends PCR or antigen testing priority for specific groups (updated 8/24/20)[1]:

1. **High Priority Symptomatics:** Hospitalized, live in congregate settings, healthcare workers, first responders, residents in long term care facilities.
2. **Priority Symptomatics:** Anyone with symptoms of potential SARS-CoV 2 infection.
3. **Priority Asymptomatic**: Individuals who are prioritized by health departments or clinicians, including but not limited to: public health monitoring, sentinel surveillance, presence of underlying medical condition or disability, residency in a congregate housing setting such as a homeless shelter or long term care facility, or screening of other asymptomatic individuals according to state and local plans.

Detection of other respiratory pathogens does not exclude SARS-CoV-2 infection as concomitant infection has been reported from below 6% to as high as 37%.[2–4]*

UPDATED CDC GUIDELINES (9/10/2020):[5]

Role of PCR testing to discontinue isolation/precautions: For all people except those severely immunocompromised, CDC no longer recommends test-based strategy *except* to discontinue isolation/precautions earlier than the 10-day period.

Role of PCR after discontinuation of isolation/precautions: For persons previously diagnosed with COVID-19 who remain asymptomatic, retesting is not recommended within 3 months after symptom onset of COVID-19 infection. Quarantine is not recommended either in the event of close contact with an infected person. If the person develops new symptoms consistent with COVID-19 during 3 months after the date of initial symptom onset and alternative etiology cannot be identified, then the person may undergo retesting. Quarantine may be recommended. For those who never developed symptoms, the date of the first RT-PCR test should be used in place.

Role of serologic testing: Serologic testing should **not** be used to establish presence or absence of COVID-19 infection or reinfection.

THE TESTS:

Classic RT-PCR assay: Can be performed in 4 hours, though most providers obtain results in 2-4 days.

Presently, diagnostic testing involves using a CDC approved real-time reverse transcription-PCR (RT-PCR) assay that targets SARS-CoV-2 nucleocapsid gene.[6] A literature review found a RT-PCR sensitivity of 56-83% for COVID-19.[7] Additionally, a meta-analysis of the current literature, pooled RT-PCR sensitivity was found to be 89% for COVID-19.[8] In a non-peer reviewed study of 87 Chinese patients ultimately diagnosed with COVID-19, RT-PCR was found to have a sensitivity and specificity of 78.2% and 98.8% respectively [9]*, while a retrospective study of 103 patients investigated for COVID-19 in China, found sensitivity of the first RT-PCR test to be 42%, which increased to 75% on the second round of testing—similar observations were seen in other studies.[9–12] Another retrospective, non-peer

reviewed study looking a 4653 close contact patients from Guangzhou found a higher sensitivity of 71.9% after first testing that increased to 92.2% with second testing, and reached 100% by sixth testing; in contrast, a specificity of 99.96% was found after initial testing.[13]* A large study of 1014 patients in Wuhan found that out of 413 negative RT-PCR tests, around 250 had chest CT findings consistent with infection and were classified as either probable or high likely cases.[14] A recent non-peer reviewed, systemic review of 5 studies consisting of 957 patients from China estimated a false negative rate ranging from 2-29% depending on a prevalence of disease between 30% and 80%. Authors noted that heterogeneity of studies, risk of bias, and other issues makes the certainty of evidence from this review very low however.[15]*

The time of diagnostic testing relative to contraction disease will influence sensitivity greatly as viral shedding dynamics change. A retrospective cohort study of a Zhejiang province patient population found median duration of virus detection from respiratory samples to be around 14 days in mild cases and 21 days in severe cases after onset of symptoms.[16] Another pooled analysis review of 1330 upper respiratory specimens from confirmed positive patients estimated false-negative rate by day since infection. It found that the probability of a false negative decreased from 100% on day 1 to 67% on day 4 and then to 38% on day 5 which was the day when symptoms would typically present themselves. False negative rate continued to fall to its lowest point on day 8 at 20% after which it increased to 21% on day 9 and to 66% on day 21.[17] Primarily, low patient viral load in early disease stages can lead to initial false negatives with other factors affecting the sensitivity including irregularity in specimen type, sampling technique, variation in detection rates of differing manufacturers, and immature development of nucleic acid detection technology.[11] RT-PCR may take up to 4 days to convert in a patient with COVID-19.[10] There is currently no evidence that persisting airway PCR fragments suggest a possibility of transmission. Overall at this time, data evaluating RT-PCR sensitivity has been variable and specificity has been limited.

It is important to note that detection of viral RNA does not confirm the presence of infectious virus. For patients with moderate symptoms infectious virus has not been retrievable after 10 days since the onset of symptoms.[18,19] However, infectious virus from a few severe cases have been found to be recoverable up to 32 days since onset of symptoms and with high cycle threshold (Ct) values.[20*,21]* Cycle threshold is the number of PCR cycles required for fluorescent signal to be detected from a given nucleic acid sample. A Ct value of 40 is typically the cut off for a positive test to be returned.[22] There has been limited research into the relationship between viral load detection by RT-PCR and transmission potential. A study from Taiwan evaluating infective potential of 60 upper respiratory clinical specimens found that samples containing higher viral loads and showing greater genome integrity tended to have a higher culture success rate. 5-6 $\log_{10}$ genome copies/ml appeared to be a reasonable viral load requirement for culture, but there were non-culturable samples that had greater than 7 $\log_{10}$ copies. Another study using a multivariate analysis found viral loads above 7 $\log_{10}$ to be associated with isolation of infectious virus from respiratory specimens.[20]* Viral genome integrity also appears to be a significant factor as there was a higher correlation of expression of structural (envelope, nucleocapsid) and non-structural (RNA-dependent polymerase) genes in culturable specimens compared to non-culturable specimens.[23]

As of July 8th, CDC recommends healthcare workers obtain a nasopharyngeal specimen, but if not possible an oropharyngeal, nasal mid-turbinate, anterior nares swab, or a nasopharyngeal/nasal wash/aspirate specimen is acceptable. A specimen study suggests lower respiratory samples have higher detection rates than upper respiratory samples, and PCR via saliva samples have been shown to detect SARS-CoV 2 in 87-92% of positive patients (n=35).[24,25] If available and if upper respiratory specimen is negative despite high clinical suspicion, a lower respiratory specimen is recommended including testing of sputum if cough is productive (induction of sputum is not recommended), a lower respiratory tract aspirate, or bronchoalveolar lavage sample.[26] In a study of specimen type detection rates, bronchoalveolar lavage showed the highest positive rates (93%), followed by sputum (72%),

nasal swab (63%), fibrobronchoscope brush biopsy (46%), pharyngeal swabs (32%), feces (29%), and blood (1%).[24] This suggests nasal swabs may be superior to oral swabs, however, only 8 nasal swabs were taken in the study versus 398 pharyngeal swabs. Another non-peer reviewed, retrospective study of 866 samples also found sputum to have the highest positive rate (74%-89%) among upper respiratory specimens, followed by nasal swabs (54%-73%) within the first 14 days since onset of symptoms.[27*]

There has been increasing evidence for utilization of saliva samples for testing. Studies have shown a possible role for using saliva for diagnosis given the advantages from the ease of collection and reduced risk to healthcare workers compared to nasopharyngeal or oropharyngeal swabs.[28] A study from Brazil consisting of 155 patients, compared both nasopharyngeal and saliva samples collected from each patient. Saliva samples had a 94.4% sensitivity and there was 96.1% agreement between the tests of the two different sample types.[29] Another study of 224 patients directly comparing nasopharyngeal and saliva samples using the CDC RT-PCR test found a 100% positive percent agreement and a 99.4% negative percent agreement.[30] Later intestinal infection has been shown in COVID-19 patients, as there was a transition between oral swab positive rt-PCR to anal and blood positive rt-PCR, suggesting that a reliance on oral swabs alone can miss infection or lead to premature discharge.[33] Though stool samples have yielded positive PCR in COVID-19 patients and have shown a longer median duration of detectability[16], there has been little evidence of isolating infectious virus from fecal samples.[18] However, one study looking at 28 fecal specimens from Guangdong patients showed that infectious virus from two out of three patients whose samples were positive could be recovered.[35] This indicates fecal-oral transmission is possible despite sparse evidence that it has played a significant role in spread.

Emerging POC RT-PCR tests[36]**:** Three rapid point-of-care (POC) diagnostics, Cepheid Xpert Xpress, Abbott ID NOW, and GenMark ePlex were recently granted emergency use authorization (EUA) and their clinical performances were determined by detection of nasopharyngeal specimens from 108 positive patients. Xpert Xpress had the lowest limit of detection (100 viral copies/ml) and also the highest positive percent agreement (98.3%) compared to reference RT-PCR test. ID NOW had the fastest result time (17 min average), but losses in analytical performance make Xpert Xpress a more appealing POC option in the future with results being returned after 46 min on average. Currently, Xpert Xpress and Abbott ID NOW are used in 36% and 24% of public health laboratories, respectively.[37]

RT-LAMP: There is limited evidence for the diagnostic efficacy of reverse transcription-loop mediated isothermal amplification (RT-LAMP) for COVID-19 including studies from China and NYC. Sensitivities of 89.9% from 248 samples and 95.6% from 201 samples respectively were reported in these studies.[4*,22] RT-LAMPs rapid amplification of the target viral sequence coupled with a colorimetric assay allows for detection of SAR-CoV-2 viral load within an hour of specimen collection. This drastically reduced sample processing time coupled with easy interpretation of test results and reduced need for high sample purity makes RT-LAMP an ideal candidate for POC diagnosis that can improve control of disease spread and reduce time to treatment. Further study with larger cohorts, and specimen samples other than nasopharyngeal and oropharyngeal swab samples that were used in the studies above is likely needed before widespread implementation. RT-LAMP was used for detection of MERS-CoV and SARS-CoV with high sensitivity and specificity as well.[39,40]

ANTIGEN DETECTION: Though results are available within minutes, this is not currently thought to be helpful when considering antigen detection for other respiratory viral pathogens, since sensitivity is suboptimal. Qidel has an antigen detection test which yields results in 15 minutes through use of their analyzer counterpart and has a self-reported positive percentage agreement of 80%.[41,42] Shenzehn YHLO Biotech Co. has utilized a IgM/IgG CLIA test kit which uses nucleocapsid (N) protein and peak protein (S). The test sensitivity in both classes of antibodies was

reported at over 90%. Using this test, authors noted that IgG class antibodies increased over time, eventually stabilizing at a high level.[43]

Studies show that antigens are generally only detected when the virus is actively replicating, therefore these tests are recommended mainly for acute or early infection. Performance of the test is heavily dependent on time from onset of illness and concentration of virus. Rapid diagnostic tests (RDTs), which include antigen detection tests, can be used at the point of care by minimally trained staff offering a diagnostic advantage. This option could be of interest for decentralized testing in low resource settings and be of use for testing asymptomatic with probable exposure to the virus. Due to the low specificity of antigen tests, RT-PCR should be used to confirm negative antigen test results in high risk individuals.[44]

CRISPR: These test results are available within 40 minutes on a point of care lateral flow strip, using a nasopharyngeal swab specimen. Thus far, CRISPR has been shown to have a 95% positive predictive value and 100% negative predictive value but is not available to public yet.[45]

SEROLOGY / ANTIBODY TESTING: The utility of serology lies primarily in surveillance and epidemiology as well as confirmation of COVID-19. A recent study reveals that positive antibody (ELISA) tests to the receptor binding domain (S1 spike protein) of SARS-CoV 2 is specific and indicative of infection, but presence of this antibody may not confer protective immunity.[46] Researchers are still trying to establish which host antibody neutralizes the virus, if any—Serum detection of such an antibody would have the most utility for clinicians and epidemiologists.
The virus gains entry through its spike glycoprotein which binds to healthy cells in humans leading to infection. Antibodies B38 and H4 block binding between the spike glycoprotein receptor binding domain (RBD) of the virus and the cellular receptor angiotensin-converting enzyme 2 (ACE2).[47] These antibodies show promise for fighting the virus and potential for a vaccine. Another study of a cohort of SARS-CoV-2 recovered patients showed that passive transfer of a nAb provides protection against disease in high-dose SARS-CoV-2 challenge in Syrian hamsters, as revealed by maintained weight and low lung viral titers in treated animals.[48] A study that mapped epitopes in the receptor binding domain found 11 neutralizing antibodies that can target this domain.[49]

Results of two case studies and one retrospective cohort study suggest that serology results are a reliable SARS-CoV 2 infection confirmation test about two weeks after illness onset, with seroconversion timelines similar to that of the 2003 SARS virus.[46,50–52] Seroconversion also correlates with symptom severity. Therefore, antibody testing during incubation or for those with early symptoms may lead to false negatives.[52] Anti-SARS-COV-2 serology has a lag time of 4 to 6 days after first initial positive rt-PCR on day 5 of exposure.[51]

The 2003 SARS infection does not fully protect from SARS-CoV 2, as only moderate cross neutralization has been shown.[53] Though host cross neutralization does occur, possibly leading to false positives from a previous SARS infection, the 2003 SARS coronavirus has not circulated the human population since 2003 and positive neutralization of this previous pandemic was found to be undetectable six years after infection, so false positives due to this cross neutralization are unlikely.[54]

While there are many commercially available testing kits, especially due to the FDA's Emergency Use Authorizations (EUA), the most reliable appear to be Abbott Laboratories IgG (EUA 4/26/20) and Roche Diagnostic's Pan-Ig (EUA 5/3/20)—Both detect antibodies to the SARS-Cov 2 N antigen. They both self-report a sensitivity of 100% after 14 days of illness onset. Specificity of the Abbott test is 99.6% and that of the Roche test is 99.8%.[55] However, monoclonal SARS-CoV-2 nucleocapsid antibodies have been generated and may be promising for a useful rapid antigen test in the future.[56]

The CDC uses a serologic test to detect SARS-CoV-2 antibodies in serum using purified SARS-CoV-2 protein as an antigen in the ELISA. It has a specificity greater than 99% and a sensitivity of 96%, and can be used to identify prior SARS-CoV-2 infections without molecular diagnostic confirmation.[57]* A serology study of patients in China with COVID-19 showed that IgG and IgM antibodies were observed as early as the 4th day after symptom onset and did comparisons between IgG and IgM sensitivity, specificity, PPV, NPV, and consistency rate.[58] IgG was more sensitive, but IgM was more specific and had a greater positive predictive value.

In a study with 285 COVID-19 positive patients, a proportion of patients with positive virus-specific IgG reached 100% approximately 17–19 days after symptom onset, while the proportion of patients with positive virus-specific IgM reached a peak of 94.1% approximately 20–22 days after symptom onset. The median day of seroconversion for both IgG and IgM was 13 days post symptom onset.[59] In a study comparing antibody levels in symptomatic versus asymptomatic patients it was found that IgG was found to be at similar levels in both categories of patients after 3-4 weeks of exposure, but a higher percentage of symptomatic patients tested positive for IgM than asymptomatic patients.[60] However, in the acute phase (period when the viral RNA can be found in a respiratory specimen) IgG levels in the symptomatic group were significantly higher than those in the asymptomatic group.

Further research on antibodies showed that the presence of Antiphospholipid antibodies (aPLs) are found in critically ill patients and contribute to coagulopathy. The study comparing the sera of critically ill and non-critically ill patients showed, “aPLs were detected in 47.0% of critically ill patients (31/66), but not in patients with non-critical conditions. aPLs emerge around 35-39 days post-disease onset. Patients with multiple aPLs displayed significantly higher incidence of cerebral infarction ($p = 0.023$)”.[61] The study recommended long term follow up of COVID patients that were found to be positive for aPLs.

Recently released guidelines from the Infectious Diseases Society of America (IDSA) recommend that serology be used for the three following reasons: 1) evaluation of patients with a high clinical suspicion for COVID-19 when molecular diagnostic testing is negative and at least two weeks have passed since symptom onset; 2) assessment of multisystem inflammatory syndrome in children; and 3) for serosurveillance studies. Data suggests that serology lacks the sensitivity to exclude the diagnosis of COVID in it’s acute phase.[62]

An article studying COVID in Iceland found that of the 1797 persons who had recovered from SARS-CoV-2 infection, 1107 of the 1215 who were tested (91.1%) were seropositive. To be considered seropositive, the study required positive results from both pan-Ig antibody assays. Antiviral antibody titers assayed by two pan-Ig assays increased during 2 months after diagnosis by qPCR and remained on a plateau for the remainder of the study thus results indicate that antiviral antibodies against SARS-CoV-2 did not decline within 4 months after diagnosis.[64]

Data regarding reinfection by COVID is still not well established but for the first time, a Hong Kong man was found to be infected twice. Whole genome sequencing was performed directly on respiratory specimens collected during two episodes of COVID-19 in a patient and was differentiated as re-infection and not persistent viral shedding. The second episode of asymptomatic infection occurred 142 days after the first symptomatic episode in an apparently immunocompetent patient. During the second episode, there was serological evidence of elevated C-reactive protein and SARS-CoV-2 IgG seroconversion. Viral genomes from first and second episodes belong to different clades/lineages.[65] Another case of reinfection in a person in Nevada was found as the viruses associated with the infections had a degree of genetic discordance that couldn’t be reasonably thought to be due to short-term evolution.(citation)

ANCILLARY DIAGNOSTICS:

CHEST IMAGING: Chest CT is thought to be more sensitive than chest x-ray, since this was shown in previous SARS-CoV infections.[66] COVID 19 CT findings (n=21) include bilateral pulmonary parenchymal ground-glass and consolidative pulmonary opacities. Several cases had a rounded morphology and a peripheral lung distribution. Notably absent were lung cavitation, discrete pulmonary nodules, pleural effusions, and lymphadenopathy.[67,68]

A retrospective study originating from Wuhan, China investigated the clinical utility of both RT-PCR and chest CT in the workup of suspected COVID-19 patients. A total of 87 patients underwent both tests and the sensitivity for CT and RT-PCR were 97.2% and 84.6% (first-round PCR) respectively. The authors concluded that patients with chest CT features of COVID-19 should be isolated and RT-PCR should be repeated at intervals of 2-3 days.[69] This study proposes the interesting idea of utilizing a chest CT as a diagnostic tool for patients that are at high risk to have contracted COVID-19 who may have an asymptomatic clinical presentation.

BIOMARKERS: Hypoalbuminemia, lymphopenia, elevated LDH, and elevated CRP were highly correlated to the acute lung injury. Age, viral load, lung injury score, and blood biochemistry indexes, albumin, CRP, LDH, and lymphocytes may be predictors of disease severity.[70]

CURRENT STATUS OF TESTING IN SAN ANTONIO? TEXAS? US?

There are currently over 40 testing sites in Bexar county with many offering drive-thru testing (PCR), four free testing sites and two mobile pop-up sites that take walk in clients.[71] This drive through method was successful in South Korea, allowing for higher testing capacity and less likely to lead to cross infection between patients.[72] When discussing the cost of tests, many of the large health insurers, including are waiving fees and coinsurance for medically necessary SARS-CoV 2 testing.[73] If a patient does not have insurance, access to testing comes at no charge.[74]

As of August 31th, 2020, Bexar county has reached the Metro Health goal of a testing capacity of 8,200 tests per day, based on estimated need for our population. As of August 31st, 2020, there have been 276,686 COVID-19 test results to date. Cases have started to decline as weekly positive test percentage has come down from 14.8% during 7/26-8/1 to 6% during 9/6-9/12. Amongst the positive 47,184 cases, 38,542 are confirmed while 8,642 are probable. The tests performed where both molecular (PCR/NAAT) and antigen (FIA) tests.[74]

TESTING IN KEY POPULATIONS:

RACE, SEX, & ETHNICITY: Angiotensin-converting enzyme 2 (ACE-2) has been identified as the receptor for SARS-CoV-2 entry, and is a point of focus for population analyses regarding viral transmission, specifically delineation of ACE-2 expression in these populations, since this may reveal a difference in infection potential.[76] The cellular serine protease TMPRSS2, secondarily, is another point of focus and plays a role in the transmission and severity of COVID-19 outcomes. TMPRSS2 has been shown to interact with the viral S protein of SARS-CoV-2; allowing viral entry into host cells by activating viral spike proteins.[77] It has been shown that TMPRSS2 transcription is regulated by androgenic ligands and an androgen receptor binding element in the promoter region for this gene.[78]

While some studies report that men have higher levels of circulating ACE-2 than women, others report higher ACE-2 serum activity in older women compared to younger women with no sex-based differences in ACE-2 expression.[79] On the other hand, evidence suggests that TMPRSS2, whose normal physiologic function remains unknown, is expressed several folds higher in the prostate compared to other human tissues and is also present in airway epithelia.[77] The involvement of TMPRSS2 in viral spike protein priming and its differential expression in prostate tissue may explain, to some degree, the higher fatalities suffered by men infection with COVID-19.[80] Early studies from China revealed equal distributions of COVID between sexes, but a recent review suggests critical diseases have a predilection to be male.[81] There were no major differences in the absolute number of confirmed COVID-19 cases in aggregate data from Italy, China, Spain, and Germany. However, equal absolute cases may point towards a higher incidence of COVID-19 in men in older age groups because there are fewer numbers of older men due to their shortened life expectancy. Hospitalizations in men exceeded those of women by 1.5-fold and case fatality rates reported in the countries listed previously are relatively homogenous and range between 1.7-1.8 when comparing men to women. This may be explained by ACE-2 and TMPRSS2 expression differences between male and female.[77] Moreover, studies have suggested that patients who self-identify as Asian express more ACE-2 receptors on their lung parenchyma, relative to White and Black populations.[82] However, it is not prudent to selectively use these factors solely to allocate screening resources, as the dynamics of COVID-19 acquisition are complex.

Surveillance data reveals that between 1,968 COVID-19 cases with information on race & ethnicity, 43.4% identified as non-Hispanic white, 32.0% identified as non-Hispanic black, 11.7% identified as Hispanic, and 12.9% identified as either other race or had unknown race status.[83] Estimates from the US Census Bureau stratify race & ethnic representation in the United States as: non-Hispanic White (60.4%), non-Hispanic Black (13.4%), and Hispanic or Latino as 18.3%. The differences above highlight differential representation in COVID-19 cases among races with their actual representation in the United States. Such differences may be due to allocation of resources, institutional differences in testing, surveillance county representation, or differences in access to care amongst race & ethnicity.

A retrospective study conducted in Philadelphia focused on an underserved area with a predominance of African American people COVID-19 positive. From this population, 49.3% of patients had acute kidney injury (AKI) in addition to COVID-19. The patients with AKI had significantly lower baseline estimated glomerular filtration rate, higher FIO2 requirement, as well as elevated D-dimer on admission. Additionally, heart failure was also consistently associated with AKI even after adjusting for eGFR in logistic regression, suggesting a potential cardiorenal component in the decline of kidney function.[84] A separate study performed in Detroit showed that male sex and age greater than 60 were significantly associated with mortality, as evidenced by odds ratios of 1.8 and 5.3 respectively. Although 72.1% of the study's 463 subjects were African American, the study reports that "African American race" is not significantly associated with mortality with an odds ratio to be 0.98.[85]

HOMELESS: The homeless population in the United States represents a proportion of individuals who experience dynamic housing situations, income, and mobility—unearthing a concern for viral transmission and testing. Crowding and suboptimal hygiene practices also pose concerns for the transmission of a respiratory virus within the homeless population.[86] A significant amount of homeless Medicaid patients rely on emergency room settings for medical care, and given the burden that these settings face in the COVID-19 pandemic within the United States, testing in these populations warrants evidence-based commentaries.[87,88]

Close-quarters of shelter for homeless populations has been notorious for expedited transmission of infectious agents.[89–91] Preliminary analyses from other cities, such as Boston, Massachusetts suggests that testing in homeless populations represents a hidden source of COVID-19 transmission and cases. Therefore, anticipation for

these populations including more accommodating testing with access to follow-up serial testing, may represent efficient utility of resources by the public health sector.[92]

Given the lack of reliable geographic stasis in the homeless population as well as a potential lack of reliable contact(s), speculation leans toward rapid-testing modalities. Quarantine or special housing accommodations in those who test positive have not been delineated.

SOCIOECONOMIC STATUS: Differential outcomes in socioeconomic status (SES) are well distributed in the literature for an array of pathology. A study of COVID-19 cases in New York City found that SES is a significant predictor of testing volume and test positivity.[93] Another study reported that those living in low income households are more likely to have conditions associated with an increased risk of contracting COVID-19 than those living in higher income households.[94] Recent reviews have suggested that health literacy may be a link between socioeconomic status and health disparities.[91] Moreover, perceptions of healthcare cost may represent barriers to patient engagement in testing initiatives. Scarcity of financial resources has been shown to impact decision making, with profound impacts in potential compliance to public health measures, such as quarantining and has the potential to exert exacerbated effects when COVID-19 testing becomes more ubiquitous and accessible.[92,95]

Testing initiatives have been made more accessible for those without health insurance in some jurisdictions in the United States. Differences in COVID-19 testing accessibility in academic healthcare institutions versus private have not been investigated and represent potential geographical constraints in testing status of those with low SES. Despite the ubiquity of testing, no formal systemic investigations have shown the effects of increased outreach on community engagement in patients with low SES.

A study that took place in New Jersey displayed that patients in lower SES brackets were more likely to present later in the disease course with already worsening shortness of breath and cough. These patients also tended to reside in areas with higher population densities. These lower SES patients also had higher levels of serum ferritin and creatinine phosphokinase, both of which are associated with in-hospital death most likely explained by seeking treatment later in the disease course.[96]

CHILDREN: Children are a population which may be overlooked due to generally more mild clinical outcomes when compared to those of the elderly. However, children should also be a group of diagnostic interest due to their ability to transmit COVID-19 past the standard 14 day isolation period while asymptomatic.[97]

In the few studies available, some children were shown to have normal T, B, and NK lymphocyte levels in contrast to that of the average adult COVID-19 patient. Additionally, in a case report originating from China, a 14-month old's lungs displayed ground glass opacities in the lower lobe of the right lung on chest computed tomography. Most notably, although clinical symptoms resolved at 9 days after symptom onset, the virus RNA in nasopharyngeal swabs and stool turned negative first at 28 days after symptom onset. This suggests that contagiousness of the patient still remains unclear despite clinical recovery.

On chest CT, consolidation with surrounding halo-sign is considered typical of pediatric patients. However, chest CT alone cannot accurately diagnose COVID-19 due to similar radiological presentations with other infections.

SURGICAL PATIENTS: Due to the possibility of an asymptomatic patient having a false negative test result, other clinical features can be used to raise suspicion for a possible COVID-19 infection. In a case report detailing a patient undergoing elective procedure, the surgical team noticed the patient had higher PEEP and FIO_2 requirements than

normal. Additionally, Trendelenburg positioning did not improve patient pulmonary compliance or PEEP requirements, prompting the team to perform a second COVID-19 test which returned positive.[98]

OTHER POPULATIONS TO MONITOR: Major targets for monitoring include locations that have already proved problematic for promoting outbreaks. These locations include schools, universities, public housing, long-term care facilities (LTCF), skilled nursing facilities (SNF), manufacturing/warehouse facilities, meat processors, maritime ships, naval vessels, indoor exercise facilities and shopping areas.[99] In Spain and Sweden, COVID-19 deaths in patients residing in LTCF have represented 66% and 49% of all COVID-19 deaths respectively by May 18, 2020, displaying the importance of continuously testing these locations.[100] In one particular study taking place in Los Angeles, California, serial testing revealed 19.7% (19/96) of the residents in a SNF tested positive for COVID-19 where 3 residents were tested due to symptoms, and 16 were asymptomatic. By utilizing serial testing, the positive patients were quickly transferred to acute care hospitals, protecting the other residents. By employing weekly serial testing, within a month the SNF had no positive cases amongst any of the residents or nursing staff.[101] For situations similar to LTCF and SNF, weekly serial testing should be considered for quick diagnosis and action to protect the lives of everyone in the residency.

TESTING CONSIDERATIONS:

REPEATED TESTING: For populations that are at significantly higher risk such as male and older populations, repeated testing should be considered especially if the initial test displays negative results. An increase in the amount of testing as well as repeated testing is seen to improve diagnosis rates, excluding patients that currently present with pneumonia. Performing three total tests per patient displayed a 1.43-fold improvement (27.9% to 39.9%) and it was seen that performing more than 3 testing administrations was not helpful for further improvement.

POOLED TESTING: Countries such as China, Germany, India and Thailand have implemented pooled testing, which allows multiple samples to be initially tested together in pools followed by testing individual samples from positive pools. This has the potential to save significant amounts of time, resources, and money, as well as aiding institutions such as schools, offices, religious organizations, and factories in reopening safely. A recent study of pooled testing from India, tested 4620 samples in 462 pools of 10 and 14940 samples in 2990 pools of 5. 61 10 sample pools returned positive and then the individual samples from these pools were tested resulting in 72 positives. The same two-step approach was done for the pools of 5 and overall this method used 76%-93% less tests compared to individual testing.[102]

Optimal pool size based on prevalence of virus has been studied and using a test that has 95-100% sensitivity, a pool size of 5 for a prevalence rate of 5% reduced expected number of tests by 57% and increased testing efficiency by 133%. With a prevalence of 10%, a pool size of 4 would be optimal with a 41% reduction in tests needed with a 69% increase in efficiency.[103] Once prevalence is above 10%, pooled testing starts to have diminishing returns. Pooling does dilute individual samples so if a positive sample with low viral load is present in a pool, there is potential for false negatives. A study showed Ct values of pools containing up to 30 samples were under 30 with the average Ct values of the pool being 5 cycles higher than the Ct values of the individual positive specimens (Ct values in the 21-29 range).[104] Detection of a single positive sample in pools of up to 32 and even 64 with additional cycles has been reported.[105] Pooling seems to not affect test sensitivity if Ct values are under 35, but in pools that had specimens with values greater than 35 a false negative rate of 13.3% was found in one study.[106] A two-fold dilution results in a 1.24 increase in the Ct value[105] so if a specimen with a Ct value > 38 is in a pool of 5 then the pool's Ct value will be over 40 resulting in a negative result.

Here in the US in mid-July, Labcorp and Quest Diagnostics received an EUA for a pooled testing method capable of testing 4-5 samples.[107,108] The State University of New York (SUNY) has received approval to started pooled testing of saliva samples in pools of 10-25 and anticipates being able to conduct at least 12,000 more tests daily.[109] Though pooled testing might be of limited use currently for many state laboratories due to test positivity rates above 10% in many states, it can still be of use in smaller settings where prevalence is found to be lower than the area it resides in such as particular districts, regions, or counties. State laboratories use their percentage of positive tests as an estimate for prevalence in their region as other methods for estimating community prevalence aren't available currently.

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