

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 10/21/20)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*} [Combined testing modalities are available such as Cepheid's point of care \(POC\) Xpert Xpress SARS-Cov-2/Flu/RSV test which received emergency use authorization \(EUA\) in October 2020 and showed 97.9% positive predictive agreement with the benchmark SARS-CoV-2 RT-PCR test.](#)⁵ [Other tests such as the CDC Influenza SARS-CoV-2 Multiplex Assay are available as well.](#)⁶

UPDATED CDC GUIDELINES (10/19/2020):⁷

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. Test based strategies are not to be used to discontinue quarantine (for people who are close contacts of someone with COVID-19 and exposed but not symptomatic).

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 for those with previously diagnosed COVID-19. 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:

BENCHMARK 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.⁸ A literature review found a RT-PCR sensitivity of 56-83% for COVID-19.⁹ Additionally, a meta-analysis of the current literature, pooled RT-PCR sensitivity was found to be 89% for COVID-19.¹⁰ 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 ^{11*}, 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.¹¹⁻¹⁴ 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.^{15*} 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.¹⁶ A preprint, systematic 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.^{17*}

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.¹⁸ 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 21.¹⁹ 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.¹³ RT-PCR may take up to 4 days to convert in a patient with COVID-19.¹² 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.

As of November 5th, CDC recommends healthcare workers obtain a nasopharyngeal specimen, but if not possible an oropharyngeal, nasal mid-turbinate, anterior nares swab, a nasopharyngeal/nasal wash/aspirate specimen, or a saliva specimen collected by supervised self-collection 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).^{20,21} 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.²² 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%).²⁰ 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.^{23*} A direct study comparing mid-turbinate and nasal swab specimen's sensitivity to nasopharyngeal specimen's sensitivity found a positive percentage agreement of 94% and 96% respectively.²⁴

Collecting saliva specimens for diagnosis has the advantages of ease of collection and reduced risk to healthcare workers compared to nasopharyngeal or oropharyngeal swabs.²⁵ 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.²⁶ 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.²⁷ 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.²⁸ Though stool samples have yielded positive PCR in COVID-19 patients and have shown a longer median duration of detectability¹⁸, there has been little evidence of isolating infectious virus from fecal samples.²⁹ 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.³⁰ This indicates fecal-oral transmission is possible despite sparse evidence that it has played a significant role in spread.

VIRAL CULTURE: 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.^{29,31} However, infectious virus from a few severe cases has been found to be recoverable up to 32

days since onset of symptoms and with high cycle threshold (Ct) values.^{32,33*} 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.³⁴ 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 log₁₀ genome copies/ml appeared to be a reasonable viral load requirement for culture, but there were non-culturable samples that had greater than 7 log₁₀ copies. Another study using multivariate analysis found viral loads above 7 log₁₀ copies/ml to be associated with isolation of infectious virus from respiratory specimens.^{32*} 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.³⁵ Another study from England assessing culturability of 324 positive samples based on Ct values found that the estimated odds ratio of recovering infectious virus decreased by 0.67 for every 1 increase in Ct value. 38/44 samples with Ct values between 20-25 were culturable compared to 5/60 samples with Ct values over 35. The temporality of infectious potential was also assessed, and it was estimated that 40.1% of samples collected 7 days after symptom onset would be culturable compared to 6% of samples collected 10 days after symptom onset.³⁶

POC RT-PCR TESTS³⁷: Three POC diagnostics, Cepheid Xpert Xpress, Abbott ID NOW, and GenMark ePlex have been granted EUA and their clinical performances were determined by detection of nasopharyngeal specimens from 108 positive patients. Xpert Xpress had the lowest limit of detection (LOD) (100 viral copies/ml) and the highest positive percent agreement (98.3%) compared to reference RT-PCR test with results being returned after 46 minutes on average. ID NOW had the fastest result time (17 min average) but had a higher LOD (20,000 copies/ml) and lower positive percent agreement (87.7%). Currently, Xpert Xpress and Abbott ID NOW are used in 29% and 26% of public health laboratories, respectively.³⁸

A meta-analysis found the Xpert Xpress assay to have a 99.4% sensitivity and 96.8% specificity and the ID NOW assay to have a 76.8% sensitivity and 99.6% specificity.³⁹

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. The FDA granted EUA for The Color Genomics SARS-CoV-2 RT-LAMP Diagnostic Assay on September 22nd, 2020.⁴¹ RT-LAMP was used for detection of MERS-CoV and SARS-CoV with high sensitivity and specificity as well.^{42,43}

ANTIGEN DETECTION: 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%.^{44,45} The Abbott BinaxNOW instrument-free antigen test received FDA EUA in late August. It detects the nucleocapsid protein antigen from nasal swab samples and also returns results in 15 minutes.⁴⁶ Test has a reported positive percentage agreement of 97.1% and negative percentage agreement of 98.5% when compared to FDA approved PCR test in symptomatic patients (within 7 days of onset).⁴⁷

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. Due to the low sensitivity of antigen tests, RT-PCR should be used to confirm negative antigen test results in high risk individuals.⁴⁸ WHO recommends that if access to RT-PCR assay is limited or turnaround times for test results are excessive to the point of limited utility, then antigen tests with a minimum sensitivity and specificity of 80% and 97% respectively can be used if the test is used within the first 5-7 days of symptom onset.⁴⁹

A perspective piece in the New England Journal of Medicine discussed the potential of rapidly expanding use of POC antigen tests to help combat community transmission.⁵⁰ Given the delay in results being returned from the benchmark clinical PCR test (often after the window of transmissibility has already closed)⁵¹ as well as the costs involved in getting the test, a rapid antigen detection test seems to be much better suited as a tool for identifying currently infected individuals including asymptomatics in the community. Rapid isolation of individuals during the window of transmissibility is paramount, and tests used for community surveillance should return results quickly and be inexpensive to allow frequent retesting. Since transmission tends to occur during the period of peak viral load⁵² the antigen tests' 100-1000 times higher limit of detection compared to benchmark PCR may not be consequential. Positive tests could be confirmed with a second test or a PCR test. In light of the CDC's estimate of a prevalence 10x higher than confirmed cases in the US⁵³ and daily incident cases being well over 100,000 as of early November, widespread use of rapid antigen tests like Abbott BinaxNOW, which costs \$5, in the community and at-home could be effective in breaking the chain of transmission.

However, concerns about antigen tests' specificity have been raised after a false positive rate of 60% was found when 39 samples tested by BD and Quidel antigen tests in nursing homes in Nevada were compared to benchmark PCR.⁵⁴ Thorough evaluation of these tests confirming adequate positive and negative agreement with benchmark PCR is necessary as well as separate evaluation of these test's performance on specimens from asymptomatic individuals before widespread use for community surveillance.

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 the public yet.⁵⁵ One study describes a method of virus detection that combines a simplified extraction of viral RNA with the benefits of CRISPR-mediated detection. This study shows a sensitivity for this test of 93.1% and a specificity of 98.5%. The test can be performed in less than an hour and can detect a smaller viral load than more traditional PCR. More data will be necessary to determine this test's efficacy and its ability to be implemented on a wider scale.⁵⁶

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.⁵⁷ 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).⁵⁸ 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.⁵⁹ A study that mapped epitopes in the receptor binding domain found 11 neutralizing antibodies that can target this domain.⁶⁰

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.^{57,61-63} Seroconversion also correlates with symptom severity. Therefore, antibody testing during incubation or for those with early symptoms may lead to false negatives.⁶³ 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.⁶²

The 2003 SARS infection does not fully protect from SARS-CoV 2, as only moderate cross neutralization has been shown.⁶⁴ 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.⁶⁵

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%.⁶⁶ However, monoclonal SARS-CoV-2 nucleocapsid antibodies have been generated and may be promising for a useful rapid antigen test in the future.⁶⁷

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.^{68*} 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.⁶⁹ 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.⁷⁰ 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.⁷¹ 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 ($p=0.023$)”.⁷² The study recommended long term follow up of COVID patients that were found to be positive for aPLs.

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 its acute phase.⁷³

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.⁷⁴

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.⁷⁵ 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.⁷⁶

In a preprint case study, an elderly patient two distinct episodes of symptomatic COVID-19 separated by 140 days in a single patient.⁷⁷ Findings suggest that poorly developed or waned antibodies against the D614 virus formed after primary infection in March were not protective against reinfection with the D614G spike variant acquired in July. These results could have important implications for the success of vaccine programs based on the Wuhan strain. In another study investigating NABs in the recovered subjects discharged from the hospital in full health a majority of the recovered subjects had raised significant NAB titers, however, there is a substantial number of recovered patients (10 out of 49) with no or low titers of NABs against the virus.⁷⁸ They concluded recovery from SARS-CoV-2 infection is not solely dependent on high NAB titers in affected subjects.

A study investigating the development of resistance against neutralizing antibodies indicated that antibody cocktails have the potential to be most effective against mutations that confer resistance. In antibody cocktails, two antibodies were chosen so as to bind to distinct and non-overlapping regions of the viral target (in this case, the RBD of the spike protein). It is unlikely that mutations at two distinct genetic sites will occur allowing for viral escape.⁷⁹

Three studies have investigated the persistence of antibodies post infection. A study of 5,882 people in Arizona showed individuals with severe disease exhibited elevated virus-neutralizing titers and antibodies against the nucleocapsid (N) and the receptor binding domain (RBD) of the spike protein. Spike RBD and S2 and neutralizing antibodies remained detectable through 5–7 months after onset, whereas α -N titers diminished.⁸⁰ A study in New York with 30,082 individuals also showed similar evidence with the vast majority of infected individuals with mild-to-moderate COVID-19 experiencing robust IgG antibody responses against the viral spike protein. Titers are relatively stable for at least a period approximating 5 months and that anti-spike binding titers significantly correlate with neutralization of authentic SARS-CoV-2.⁸¹ A third study showed a slightly reduced period of antibody persistence; longitudinal analysis revealed that anti-SARS-CoV-2 IgA and IgM antibodies rapidly decayed, while IgG antibodies remained relatively stable up to 105 days post symptom onset in both serum and saliva. This study was done in Ontario Canada with n=439 (serum) and n=128 (saliva) patients with COVID-19.⁸² Another discovery showed SARS-CoV-2 spike glycoprotein (S)-reactive antibodies were detectable by a flow cytometry-based method in SARS-CoV-2-uninfected individuals and were particularly prevalent in children and adolescents. The journal, Nature, highlighted the research and summarized that 5% of 302 uninfected adult participants had antibodies that recognize SARS-CoV-2, and so did more than 60% of uninfected participants aged 6 to 16.⁸³

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.⁸⁴ 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.^{85,86}

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.⁸⁷ 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.⁸⁸

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

There are currently over 80 testing sites in Bexar county with many offering drive-thru testing (PCR), 24 of them being drive-thru testing or walk up (PCR).⁸⁹ This drive through method was successful in South Korea, allowing for

higher testing capacity and less likely to lead to cross infection between patients.⁹⁰ 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.⁹¹ If a patient does not have insurance, access to testing comes at no charge.⁹²

As of November 29th, 2020, San Antonio/Bexar county has reached the Metro Health goal of a testing capacity with capacity currently 2x higher than current need. As of November 23rd, 2020, there have been 594,153 COVID-19 test results to date. Incident cases have increased as the positive test percentage rose from 8.4% during 11/1-11/7 to 10% during 11/15-11/21. Amongst the positive 80,057 cases, 66,492 are confirmed while 13,565 are probable. The tests performed are both molecular (PCR/NAAT) and antigen (FIA) tests.⁹²

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 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.⁹³ [Another study in Israel tested 133,816 samples over a 5 month period in either 8-sample or 5-sample pools depending on the changes in prevalence rate. 76% of PCR tests were spared compared to an individual testing method with minimal losses in sensitivity \(average Ct increase of 2.9 compared to positive sample Ct value\).](#)⁹⁴

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.⁹⁵ 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).⁹⁶ Detection of a single positive sample in pools of up to 32 and even 64 with additional cycles has been reported.⁹⁷ 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.⁹⁸ A two-fold dilution results in a 1.24 increase in the Ct value⁹⁷ 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.

[A study assessing the Xpert Xpress POC assay that used sample pools of 3 and 6 found all dilutions with a viral load above 60 copies/ml were detected which is consistent with the assay's 100 copy/ml limit of detection for individual testing. Strong sensitivity shown by this study makes it a viable option for pooled testing.](#)⁹⁹

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.^{100,101} 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.¹⁰² [A report of Duke University's COVID-19 prevention strategy showed the potential of pooled testing for surveillance testing. 68,913 tests were conducted on a population of 10,265 students over a two-month period resulting in 84 individuals testing positive. half of which were from asymptomatic. 59,476 of the tests done were in the form of 5-sample pools where the samples were collected from weekly/biweekly tests of asymptomatic students, and 29 of the 84 positive](#)

cases (34.5%) were identified through this pooled testing method.¹⁰³ 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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