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COVID-19 Diagnostic Testing

Nucleic acid amplification testing (NAAT) for COVID-19 detects RNA from SARS coronavirus 2 (SARS-CoV-2) and is the primary test used for diagnosis of acute infection. NAAT methods such as **Reverse Transcription – Polymerase Chain Reaction (RT-PCR)** are considered the gold standard for respiratory virus testing, with very high analytical sensitivity (the ability to detect virus from known positive and negative controls in the lab, generally >95% over numerous studies (1). Limits of detection may vary somewhat between different test methods, but all FDA Emergency Use Authorization (EUA)-approved tests for SARS-CoV-2 are expected to detect levels of virus that are present during acute symptomatic infection.

Clinical sensitivity of RT-PCR varies by site of sampling, likely due to variation in quality of sampling technique, time of sampling with respect to disease course (viral titers are highest early in infection (2,7), and variation in the distribution of virus in the upper versus lower respiratory tract. Our understanding of clinical sensitivity of RT-PCR is based on a) prior studies using RT-PCR to detect other respiratory viruses (1), and b) limited data on SARS-CoV-2 (2–4). Prior studies of respiratory viruses have found that sampling by nasopharyngeal (NP) swab may be more sensitive than oropharyngeal (OP) swab sampling, and that a combination of NP + OP may increase sensitivity, although variation by virus was observed (4–5). Two limited studies of SARS-CoV-2 have compared percent test positivity based on sampling site over the disease course but were not done in a way that allowed accurate calculation of sensitivity. The larger study (213 patients, not-yet peer reviewed) found a decrease in viral detection over time, with test positivity in the first 14 days after symptom onset somewhat higher in NP swabs (72%) versus OP swabs, and with lower respiratory samples remaining positive in severe disease (2) . The smaller study (9 patients) found high viral loads in pharyngeal samples during the first week of symptoms, with 100% test positivity during the first five days of symptoms and 46% test positivity after the first five days, independent of swab type (7). While more information is needed and there likely is individual variation in the time course of viral shedding, existing studies suggest that clinical sensitivity is likely very high during the early acute, symptomatic phase of infection, but lower during later stages of infection when viral titers may be low. No data on test sensitivity in asymptomatic infected individuals exists. Additional studies suggest that older patients have higher viral loads and that sputum and lower respiratory specimens (endotracheal aspirate) have high viral loads in patients with severe pulmonary disease (7,8). A recent study (not yet peer-reviewed) suggests that patient self-sampling of the anterior nares or mid turbinate nares may have comparable sensitivity to that of a clinician administered NP swab (10).

What does a negative RT-PCR test mean? The negative predictive value (NPV) is the probability that a person with a negative test does not have infection and differs depending on the prevalence of disease in the population. The prevalence of active SARSCoV-2 viral infection in the Bay Area and in the U.S. is changing over time (https://covid19.ca.gov/data-and-tools/). When the estimated prevalence of asymptomatic patients in the Bay Area is low, the negative predictive value for testing an asymptomatic

Main points

1.RT-PCR is the diagnostic test of choice

2.Sensitivity is high early in symptomatic infection but decreases over time

3.Recommended specimens:

- Combined NP swab + OP swab
- Combined Mid turbinate + OP swab
- Tracheal aspirate (intubated patients)
- Anterior nasal swab (asymptomatic patients with frequent screening after initial negative)

4.When to use serology:

- Patients with negative PCR testing strongly suspected to have COVID-19 -documentation of seropositivity for plasma donation or vaccine studies

patient prior to surgery is very high. For example, if the prevalence is assumed to be 1% and the sensitivity/specificity of a NP+OP swab test is estimated at 80%/98% (sensitivity estimate based on sampling at an unknown time over the entire course of infection), then the negative predictive value of the test is 99.8%. When the overall prevalence rises or for higher suspicion cases such as symptomatic patients or those with known exposures, the negative predictive value declines. For example, when the prevalence is 10%, the negative predictive value is estimated to be 97.2%.

Point of Care (POC) COVID Testing for active infection

Rapid point of care tests for active COVID infection include molecular and antigen based detection systems. The clinical performance of these POC tests for active COVID infection largely depends on the circumstances in which they are used and the technology used in the rapid assay system. POC molecular-based COVID assays are considered more sensitive than POC antigen-based COVID assays, however neither are as sensitive to detection of low viral loads as main lab NAAT / RT-PCR testing. Rapid tests are particularly helpful if the person is tested in the early stages of infection with SARS-CoV-2 when viral load is generally highest. They also can be informative in diagnostic testing situations in which the person has a known exposure to a confirmed case of COVID-19. Rapid tests may be informative when used for screening testing in high-risk congregate settings in which repeat testing could quickly identify persons with a SARS-CoV-2 infection to inform infection prevention and control measures, thus preventing transmission throughout the congregate setting. In this case, there may be value in providing immediate results with rapid POC even though they may have lower sensitivity than NAAT tests, especially in settings where a rapid turnaround time is required.

The specificity of rapid POC COVID tests is generally as high as RT-PCR –which means that false positive results are unlikely. Positive and negative predictive values of all in vitro diagnostic tests vary depending upon the pretest probability of the patient being tested. Pretest probability is impacted by the prevalence of the target infection in the community as well as the clinical context of the recipient of the test.

The “gold standard” for clinical diagnostic detection of SARS-CoV-2 is main lab NAAT testing. Thus, it may be necessary to confirm a rapid POC test result with main lab testing, especially if the result is inconsistent with the clinical context. When confirming a rapid POC test result with a main lab test, it is important that the time interval between the two sample collections is as close in time as possible, and there have not been any opportunities for new exposures between the two tests. If too much time as elapsed, or there have been opportunities for new exposures between the two tests, the main lab test should be considered a separate test – not a confirmatory test.

a) **POC Abbott ID NOW Molecular COVID testing** is an FDA EUA approved rapid molecular in vitro diagnostic test utilizing isothermal nucleic acid amplification technology intended for the qualitative detection of nucleic acid from the SARS-CoV-2 viral RNA in upper respiratory sample swabs from individuals who are suspected of COVID-19 infection. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection. Studies performed on symptomatic patients soon after symptom onset suggest the ID NOW performs best in patients tested earlier post symptom onset and are approximately comparable to main lab results during this time frame. Several published and ongoing studies of symptomatic patients demonstrated that ID NOW test performance of 75-94.7% sensitivity and 96-100% specificity compared to lab-based PCR reference tests (PMID: 32327448). These studies therefore indicate that the POC ID NOW platform detects most COVID patients with higher viral loads. In contrast, the sensitivity to

detection of SARS-CoV-2 using the ID NOW POC assay is decreased relative to main lab tests in patients with low viral load. Analytically, the limit of detection of the POC ID NOW COVID assay is approximately an order of magnitude lower than main lab based methods, on average, based on the manufacturer's published genomes/reaction data and published studies (PMID: 32651238). Clinical studies of patients with low viral loads exhibit down to 35% sensitivity (65% false negatives) on the ID NOW vs main lab testing when collected using the protocols recommended by UCSF (12, 13). This data highlights that negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be tested with main lab testing.

b) **Point of Care COVID Antigen Testing** are FDA approved EUA rapid immunoassays which detect viral antigen (eg. SARS-CoV-2 nucleocapsid protein antigen). Several manufacturers produce testing kits. COVID antigen is generally detectable in upper respiratory specimens during the acute phase of infection. Manufacturer studies performed using patient samples between 0-5 days post-symptom onset (high viral loads) demonstrates 84-96% positive predictive agreement and 100% negative predictive agreement compared to main lab NAAT testing. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. The sensitivity to detection is lower in patients with low viral loads; the limit of detection of the antigen tests is several orders of magnitude higher than main lab NAAT testing (14, 15). Studies have shown that antigen levels in some patients who have been symptomatic for more than five days may drop below the limit of detection of the test. This may cause the test to return a negative result, while a more sensitive test, such as RT-PCR, may return a positive result. For this reason, antigen testing is better suited as a screening test. Negative results, from patients with symptom onset beyond five days, or in asymptomatic patients, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.

Serologic Testing detects antibodies to SARS-CoV-2 produced by the patient. Antibodies may not reach levels high enough to detect until 7-14 days after infection (7) and may never be detected in patients with impaired immunity. There is significant variability in commercial serologic assays and some point-of-care assays may have high false positivity rates due to cross-reactivity with 4 common seasonal coronaviruses that circulate widely in the United States. We don't yet know how antibody detection using certain assays correlates with protective immunity. The primary uses of serologic testing at UCSF are: 1) to improve sensitivity for COVID-19 diagnosis in patients with a high suspicion of COVID-19 but negative NAAT testing presenting more than a week into infection and 2) to document antibody response in patients planning to donate convalescent plasma or participating in vaccine trials.

Clinical Evaluation Guide for Diagnosis and Testing of COVID-19:

https://infectioncontrol.ucsfmedicalcenter.org/sites/g/files/tkssra4681/f/COVID19%20Clinical%20Evaluation%20Guide.pdf

UCSF Inpatient Adult COVID-19 Interim Management Guidelines:

https://infectioncontrol.ucsfmedicalcenter.org/sites/g/files/tkssra4681/f/UCSF_Adult_COVID_draft_management_guidelines.pdf

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