Testing for Severe Acute Respiratory Syndrome–Coronavirus 2: Challenges in Getting Good Specimens, Choosing the Right Test, and Interpreting the Results

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Objectives: We explore ways to reduce errors in laboratory diagnosis of severe acute respiratory syndrome–coronavirus 2 infection by considering preanalytic, analytic, and postanalytic sources. To address preanalytic challenges, we first consider alternative anatomic sites for specimen collection, then discuss self-collection, alternative sampling devices, and transport media. Strengths and limitations of various analytic test systems are considered in the context of postanalytic challenges associated with making test results meaningful, specifically considering the complex relationship between “positive” test results and reproduction and shedding of intact virus. Finally, we provide recommendations regarding healthcare worker surveillance and release of patients with coronavirus disease 2019 from isolation.

Data Sources: Material was derived from a Webinar available to the public, manufacturer’s websites, U.S. Food and Drug Administration, and Centers for Disease Control and Prevention websites and from both peer-reviewed papers identified by PubMed search and nonpeer-reviewed papers posted on Biorxiv and Medrxiv. Unpublished data came from the Washington State Department of Health.

Study Selection: We included studies that compared diagnostic performance strategies without introducing bias due to use of an imperfect gold standard. Case series and case reports were included as necessary to illuminate the significance of results.

Data Extraction: Data were extracted manually.

Data Synthesis: Sensitivity, specificity, and CIs were computed from article data using a composite reference standard. Nucleic acid-based tests were assumed to perform at 100% specificity.

Conclusions: Although sputum and bronchoalveolar lavage samples provide the highest diagnostic sensitivity for severe acute respiratory syndrome-coronavirus 2, nasopharyngeal, mid turbinate, and nasal specimens are suitable in most cases and require less use of personal protective equipment. When desired sampling materials are unavailable, alternatives may be substituted with no loss of performance. Both reverse transcriptase polymerase chain reaction tests and rapid nucleic acid-based tests offer good performance in most circumstances. Testing is not required to release most patients from isolation. (Crit Care Med 2020; 48:1680–1689)

Key Words: coronavirus disease 2019; sampling; severe acute respiratory syndrome–CoV-2; swab; testing

Rapid and accurate diagnosis of severe acute respiratory syndrome (SARS)–CoV-2 infection (coronavirus disease 2019 [COVID19]) facilitates treatment, reduces the spread of disease, safeguards healthcare personnel, and optimizes use of personal protective equipment. Most diagnostic testing for SARS-CoV-2 in the United States uses nucleic acid amplification tests (NAATs), particularly tests based on real-time reverse-transcription polymerase chain reaction (RT-PCR), that are sensitive and specific for detecting viral RNA. A recent review provides details of how laboratories perform NAAT and serologic testing (1).

SARS-CoV-2 testing failure often results from preanalytical deficiencies. Failing to obtain and transport an adequate specimen may lead to a false-negative result that may or may not be detectable in the laboratory. Analytical error in the laboratory will give a false-negative result if an assay fails to detect low amounts of viral RNA that should be within the limit of detection. Postanalytical interpretive failures may result from failure to understand the complex biology of SARS-CoV-2 infection, especially because the relationship between the presence of viral RNA and the presence of competent, transmissible virus is complex and incompletely understood. The speed at which SARS-CoV-2 infection has spread has only exacerbated these challenges.
The preanalytic and analytic logistical and organizational challenges are by now well-known. Sample collection has been hindered by not only a lack of supplies for testing but also by a lack of certainty as to the best anatomic sites for specimen acquisition. Similar logistical challenges have forced laboratories to adapt tests to the availability of reagents or to limit test availability, leading in some cases to slow turnaround times. In the postanalytic phase, the clinician is faced with further complexity because the results of laboratory tests based on different laboratory protocols and amplification platforms may not be equivalent and because the clinical implications of a test result depend on when in the course of disease the specimen is obtained, from which anatomic site, and on the viral load estimated by the laboratory. These diagnostic challenges are exacerbated when one considers the appropriate use of antigen and serologic tests.

In this review, we analyze currently available approaches to specimen collection, laboratory analysis (including testing platform speed and sensitivity), and result interpretation. We focus on systems available in the U.S. under Food and Drug Administration (FDA) Emergency Use Authorization (EUA). We consider the influences of time after exposure and of clinical disease course on the results of SARS-CoV-2 laboratory tests. Unlike the usual review, much of the data which we will include in this review originates in the “preprint” literature found at bioRxiv.org and medRxiv.org. Although this cited work has not been formally peer-reviewed, we provide sufficient context for the reader to draw conclusions regarding the reliability of our opinions and hope that the current shortcomings of knowledge and understanding will stimulate ongoing engagement between patient-facing clinicians and those practicing laboratory medicine (2) on all the issues surrounding SARS-CoV-2 testing. Only through such ongoing engagement can laboratory testing optimally contribute to reducing the morbidity and mortality associated with the current COVID19 pandemic.

**THE PREANALYTIC PHASE: SAMPLE ACQUISITION: SITE, SWABS, AND TRANSPORT**

**Sample Site**

The goal of sampling in a symptomatic patient is simple—maximize the likelihood of obtaining a true-positive result while minimizing both the risk to healthcare personnel and patient discomfort. This requires considerable judgment by the clinician, since there are ongoing shortages in clinic testing supplies, laboratory reagents and assays, and because the data available to inform our judgment are quite limited.

An early report from China (Table 1) provides limited insight into the relative value of sampling various anatomical sites (8). However, the interpretation is complicated by the fact that while pharyngeal swabs (which are not clearly defined as oropharyngeal or nasopharyngeal) were obtained during the first 3 days after presentation, other specimens were taken at any time during the illness. There were also a very limited number of simultaneously acquired specimens leaving considerable uncertainty as to whether these data are comparable.

The timing of sample acquisition is important. It typically takes about 5 days between first infection and the onset of symptoms; during the first part of this presymptomatic period, the odds are against obtaining a positive RTPCR result (9). Viral loads in both the upper respiratory tract (URT) and lower respiratory tract (LRT) begin to drop beginning within a week after symptom onset, with LRT viral burden significantly higher than that of the URT (10, 11). As illness progresses, virus is less likely to be cultured (12), and it is increasingly difficult to obtain a positive RTPCR result (9). Not surprisingly, studies comparing the use of pharyngeal specimens with sputum or bronchoalveolar lavage (BAL) fluid tend to show higher positivity rates for the LRT (Table 2), although significant confounding occurs since patients with more severe disease are more likely to
have BAL fluid or sputum analyzed than patients with mild disease. Nevertheless, greater sensitivity for LRT specimens than for pharyngeal specimens characterizes both mild and severe disease at all times after symptom onset (13, 14).

Viral samples from the URT have historically been acquired using a nylon flocked nasopharyngeal swab with the swab placed in viral transport media (VTM) for transport to the laboratory. Several websites provide detailed instructions on how to perform this procedure (19–21). The utility of samples obtained in this way has been compared with that of other URT sampling approaches (Table 3). Nasopharyngeal and nasal samples are more likely to yield positive results than are oropharyngeal samples. Samples taken from the mid turbinate, nose, saliva, and tongue give results that are similar to those of nasopharyngeal specimens although some authors (22) have reported that saliva has higher SARS-CoV-2 RNA concentrations in many patients. Regardless of the site selected for sampling, it is critical to obtain a good sample, since a poor sample may lead to false negative findings (23). “Loading your swab” with a good sample by using a single swab, and collecting from both nostrils or from more than one URT site (e.g. nasal and mid turbinate) is likely to improve yield. Both left and right nostrils should be sampled when collecting from the anterior nares or mid turbinate, as upper respiratory virus concentrations can vary from one side to the other (24). In both ICU and non-ICU patients, RTPCR remains positive longer in nasal swabs than in saliva, and both sites tend to remain positive for 3–6 days longer in critically ill patients (25).

SARS-CoV-2 RNA is only occasionally amplified from blood (8, 18) although one study of hospitalized patients found as many as 88% of ICU patients, and 67% of hospitalized non-ICU patients had positive blood specimens (25). RNA detected in the blood has been associated with higher rates of ICU admissions, mechanical ventilation, and 30-day mortality (33) but does not seem to indicate the presence of infectious virus (34). Urine and central spinal fluid are also unrewarding as an initial source for positive samples (35, 36). Stool specimens are frequently positive and may shed viral RNA long after resolution of symptoms; the clinical significance of this finding is unclear (37–41).

### Swabs and Transport Media

Many people view nasopharyngeal samples obtained with a nylon “flocked swab” transported in VTM as a “gold standard” for upper respiratory sampling, as this combination has worked well for a wide variety of respiratory pathogens in both children and adults. Both flocked swabs and VTM have been in short supply during the COVID19 pandemic. To obviate these problems, clinicians and laboratories have evaluated different materials (flocked vs foam vs spun polyester swabs) and different transport media (VTM vs saline vs dry swabs).

Many swab types are capable of demonstrating high performance, including traditional flocked nylon swabs, rayon swabs, spun polyester swabs, and cotton swabs (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/CCM/F737). A comparison of six different types of swabs from 10 patients, suggests that nonsterile cotton-head plastic shaft swabs transported in 95% ethanol perform comparably to nasopharyngeal swabs transported in VTM. Three-dimensional printing may produce suitable swabs (42, 43). Three-dimensional printing allows fast and easy creation of multiple swabs but is more expensive than plastic injection molding. The FDA has issued standards for evaluating 3D printed swabs, and an open-source program for creating reliable swabs is available through GitHub (44). Swabs should not be made with calcium alginate, commonly used on swabs for wound care because this substance can inhibit the polymerase chain reaction (PCR), leading to invalid results (45).

Minimum essential medium (MEM), phosphate buffered saline (PBS), and 0.9% saline are suitable alternatives to VTM for several different RTPCR assays (46–48). Dry polyester swabs work nearly as well in some assays as foam swabs transported in VTM (48). Nevertheless, not all swabs perform well with all test platforms and all transport media. Abbott, for example, warns that rayon swabs and VTM are incompatible with good performance on the ID NOW (Abbott Diagnostics Scarborough Inc., Scarborough, ME) platform (49) and recommends the use of dry swabs analyzed within 2 hours of collection. Some transport media, in particular PrimeStore molecular transport medium (EKF Diagnostics, Penarth, Cardiff, United Kingdom), contains guanidine thiocyanate, which reacts with bleach to release cyanide.
TABLE 2. Performance of Upper airway (Nasal, Oropharyngeal, or Nasopharyngeal) Swabs, Sputum, and Bronchoalveolar Lavage Specimens for Coronavirus Disease 2019 Diagnosis

| Upper Airway | Sputum | Bronchoalveolar Lavage Fluid |
|--------------|--------|-----------------------------|
| Positive/CP; Sensitivity (%) (95% CI) | Positive/CP; Sensitivity (%) (95% CI) | Positive/CP; Sensitivity (%) (95% CI) |
| 63/67; 94 (86–98) | 58/61; 95 (87–98) | (15) |
| 23/46; 50 (36–64) | 40/46; 87 (74–94) | (16) |
| 75/104; 72 (63–80) | 126/398; 32 (27–36) | 14/15; 93 (70–99) | (8) |
| 43/114; 38 (29–47) | 44/71; 62 (50–72) | (17) |
| 14/38; 36 (23–53) | 29/38; 76 (61–87) | (13) |
| 99/205; 48 (42–55) | 106/140; 76 (68–82) | 23/26; 88 (71–96) | (14) |
| 180/472; 38 (34–43) | 148/304; 49 (43–54) | (18) |

CP = composite positive.

*Multiple pharyngeal and sputum samples were taken; the data in reflect patients who at any time demonstrated a positive result from the specimen type.

Specimens were tested using both droplet digital reverse transcriptase polymerase chain reaction (RTPCR) and ordinary RTPCR methods; the results shown reflect ordinary RTPCR.

The first RTPCR tests for SARS-CoV-2 were developed by the U.S. Centers for Disease Control and Prevention (CDC), the China CDC, and the Charité Hospital (for the World Health Organization [WHO]). The primers, probes, and performance characteristics of these assays differ (Supplementary Fig. 1, Supplemental Digital Content 1, http://links.lww.com/CCM/F737). All primers and probes from the China and United States CDC amplify and detect SARS-CoV-2 with high sensitivity. The RNA-dependent RNA polymerase (RdRp) primer/probe set from the Charité hospital is significantly less sensitive. Since the RNA sequence for SARS-CoV-2 was not known at the time the Charité assay was developed, assay developers at Charité designed this primer/probe set to assure that the WHO assay would detect any bat coronavirus, regardless of sequence. This design results in loss of sensitivity for the RdRp set by several logs (50). Subsequent test development has relied both on the targets used in these three assays and additional targets.

Commercial assays used in the United States perform at a level similar or better than that of the U.S. CDC assay although design variations influence sensitivity when very low levels of viral RNA are present. All EUA RTPCR platforms have similar clinical sensitivity (Supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/CCM/F737) (51, 52). With the exception of the Abbott ID NOW system and the Cepheid Xpert Xpress systems (Cepheid Inc., Sunnyvale, CA), all available NAAT systems are restricted to high complexity laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).

RNA extraction kits have had periods of limited availability during this pandemic. RTPCR assays can be conducted without initial RNA extraction although there is a possible loss of sensitivity for low viral loads (53–56).

**The ANALYTIC PHASE: DIAGNOSTIC TESTS AND TEST PLATFORMS**

**NAAT Assay Platforms**

Laboratory diagnosis of symptomatic COVID19 patients typically uses RTPCR (reverse-RTPCR) although other techniques, including isothermal amplification, clustered regularly interspaced short palindromic repeats (CRISPR)–based testing, and antigen tests, are available. Their performance characteristics vary significantly. Results are typically reported as positive or negative, and a cycle threshold (Ct) value may be provided for each target RNA sequence. The Ct (the cycle number at which the fluorescence from a quantitative PCR reaction rises significantly above background) changes inversely with viral load—a low Ct value corresponds with a high viral RNA concentration. The relationship between Ct value and RNA concentration is complex and is not standardized between different assay systems. The absolute Ct value depends on the viral gene target and the assay system. Ct values from one manufacturer’s assay system cannot be extrapolated to another. Furthermore, the Ct value may depend on the efficacy of sampling, the volume of the transport media resulting in dilution of viral RNA, viral RNA degradation prior to laboratory analysis, or the ability to liberate viral RNA from the swab/sampling device.

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**Rapid NAAT Diagnostics**

The isothermal nicking enzyme amplification reaction (57) assay from Abbott, ID NOW, which accepts only a single
sample at a time, has the shortest time to result of any EUA systems (~15 min).

The sensitivity of this assay has been reported to be between 60% and 95% as compared with laboratory based RT PCR assays (Supplementary Table 2, Supplemental Digital Content 1, http://links.lww.com/CCM/F737). The lower sensitivity is apparently due to a significantly higher limit of detection (58, 59). Whether this results from using RdRP as an amplification target (similar to the reduced sensitivity of this target in the Charité/WHO assay) or comes from a different design feature is unclear. Swabs for this assay should be transported dry, since transport in VTM gives significantly worse results (60, 61). Depending on the prevalence of infection in the tested population, the ID NOW system may be useful for screening patients in need of emergent treatment because it is a “waived test” available for point-of-care (POC) use.

The Cepheid Xpert Xpress is also a waived test available for POC use. It has a somewhat longer time to first result than the ID NOW assay (~45 min) but is still very fast and is among the most sensitive RTPCR assays (Supplementary Table 2, Supplemental Digital Content 1, http://links.lww.com/CCM/F737).

The Sherlock CRISPR SARS-CoV-2 kit (Sherlock Biosciences, Cambridge, MA) (62, 63) uses reverse-transcriptase loop-mediated amplification (64), followed by transcription of the amplified DNA to activate the cleavage activity by a CRISPR complex. This liberates fluorescent nucleic acid reporters which may be detected using a plate reader, like those used in enzyme-linked immunosorbent assays or using lateral flow assays. Amplification can be carried out in an ordinary water bath or on a heating block. The Instructions for Use (62) claim an analytical sensitivity of 100% (95% CI 84–100%) and specificity of 100% (95% CI 89–100%) based on contrived samples (no clinical samples were validated). A similar, but not identical, assay validated on 83 patient samples (65) showed sensitivity of 95% (95% CI 84–99%) and specificity of 100% (95% CI 92–100%). The time to result is approximately 40–70 minutes, depending on whether results are obtained using a plate reader or lateral flow assay system. Use of this system is restricted to CLIA high complexity laboratories.

### Antigen Tests

NAAT assays are molecular assays that detect RNA or DNA, whereas antigen assays detect viral proteins. Numerous kits are

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**TABLE 3. Performance of Pharyngeal, Nasal and Oral Sampling**

| Oropharyngeal | Nasopharyngeal | Mid Turbinate | Nasal | Saliva | Tongue |
|---------------|----------------|---------------|-------|--------|--------|
| **Positive/CP; Sensitivity (%) (95% CI)** | **Positive/CP; Sensitivity (%) (95% CI)** | **Positive/CP; Sensitivity (%) (95% CI)** | **Positive/CP; Sensitivity (%) (95% CI)** | **Positive/CP; Sensitivity (%) (95% CI)** | **Positive/CP; Sensitivity (%) (95% CI)** |
| 136/162; 84 (78–89) | 150/162; 93 (88–96) | 19/21; 90 (71–97) | 18/21; 86 (65–95) | 23/29; 79 (62–90) | 19/29; 66 (47–80) |
| 16/17; 94 (73–99) | 17/17; 100 (82–100) | 49/51; 96 (87–99) | 48/51; 94 (84–98) | 46/51; 90 (79–96) |
| 99/205; 48 (42–55) | 311/490; 63 (59–68) | 50/52; 96 (87–99) | 48/51; 94 (84–98) | 46/51; 90 (79–96) |
| 126/398; 32 (27–36) | 5/8; 63 (31–86) | 37/37; 100 (91–100) | 33/37; 89 (75–96) |
| 99/205; 48 (42–55) | 311/490; 63 (59–68) | 50/52; 96 (87–99) | 48/51; 94 (84–98) |
| 26/36; 72 (56–84) | 27/36; 75 (59–86) | 24/36; 72 (56–84) | 24/36; 72 (56–84) |

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M D’Angeli, D Lindquist; Washington State Department of Health, Personal Communication, 2020

(26)

(27)

(28)

(29)

(30)

(31)

(32)

**Notes**

CP = composite positive.

a The authors refer to specimens as nasal swabs, but it is possible that they are nasopharyngeal swabs instead.

b Samples not necessarily contemporaneous; data may be more subject to bias.
Serological Tests
Serology relies upon the detection of the antibody response to an infectious organism. Serological tests for SARS-CoV-2 rely upon detection of antibodies that are specific to either the nucleocapsid or spike protein of the virus. In most patients, the immunoglobulin M (IgM) response begins 6–14 days after infection, and an IgG response begins shortly thereafter. Some patients may not exhibit a detectable antibody response, and others may have a significantly delayed response despite resolution of symptoms (67). The IgM response may fade, but an immunoglobulin G (IgG) response can usually be detected for weeks to months after infection has resolved. Many serologic tests have been marketed in the United States; the performance characteristics reported for this assay are based upon artificially constructed specimens, rather than clinical specimens, and there has been no independent validation.

Virus Culture
Due to the highly contagious nature of SARS-CoV-2 and the relative ease of diagnosis using molecular methods, virus culture should not be used as a routine diagnostic technique. When viral culture is employed, it must be carried out in a Biosafety Level 3 laboratory. Data from virus culture studies may ultimately be useful in developing guidelines for determining when an individual is likely to be infectious.

THE POST-ANALYTIC PHASE: PUTTING TEST RESULTS IN CONTEXT
Although RTPCR tests for SARS-CoV-2 have extremely high analytical sensitivity and nearly perfect specificity, many patients who are ultimately diagnosed with SARS-CoV-2 infection do not have a positive test on initial testing. Estimates of clinical sensitivity conducted using nasopharyngeal swabs vary widely, ranging from about 60–96%, depending on the clinical context (6, 78). Since eligibility to receive potentially helpful therapy may depend on documenting positive laboratory results, retesting is appropriate for clinically suspicious RTPCR-negative patients and may be appropriate for ongoing surveillance (see below).

Data from CDC (Supplementary Fig. 2A, Supplemental Digital Content 1, http://links.lww.com/CCM/F737) show that when RTPCR shows low levels of virus, the ability to recover culturable virus is lost; this suggests that in some patients, low PCR signals may correspond to loss of infectivity—a perspective which is supported by observational study (79). This dovetails with the observation that as illness due to SARS-CoV-2 progresses, the ability to culture virus is lost (Supplementary Fig. 2B, Supplemental Digital Content 1, http://links.lww.com/CCM/F737), just as the ability to identify the virus in respiratory specimens by RTPCR is lost (10, 80). It is possible, therefore, that the threshold Ct for infectivity will prove to be lower than that currently used for diagnosis (81). Unfortunately, there is no way at this time to determine clinically for certain when infectivity has ceased for a person suffering from COVID19. There is no data to suggest that most patients need to be tested to assess the resolution of disease or discharge from isolation. A test-based strategy is rarely appropriate for discontinuing transmission-based precautions; a symptom-based strategy should generally be used. For persons with mild to moderate COVID-19 illness, isolation and precautions can be discontinued if 10 days have passed since symptom (both respiratory and nonrespiratory) onset, so long as the patient has been afebrile for at least 24 hours without using antipyretic drugs, and other symptoms have improved. For totally asymptomatic patients who are not severely immunocompromised, precautions and isolation may
be discontinued when at least 10 days have passed since the date of the first positive test for SARS-CoV-2. For patients with severe illness, isolation, and precautions can generally be discontinued after symptoms (cough, shortness of breath) have improved, at least 24 hours have passed without fever or use of antipyretic drugs, and at least 20 days have passed since symptoms first appeared. A test-based strategy may be considered for those who are severely immunocompromised (12).

Some individuals who demonstrate serologic response after recovering from SARS-CoV-2 infection are found to be RTPCR-positive many weeks after resolution of symptoms and sequentially negative RTPCR results (82). Follow-up of 285 re-RTPCR-positive individuals from South Korea, 45% of whom were symptomatic, found no evidence of SARS-CoV-2 infectivity in these patients. Viral cultures were unsuccessful in all 108 of 108 cases, and Ct values were high, indicated low viral loads. Although it is possible that recurrent positivity reflects persistent low-level, nontransmissible infection, recurrent RTPCR positivity does not seem to constitute evidence of reinfection.

Although recovery is typically accompanied by cessation of viral shedding, people who are immunocompromised by disease or medication may have prolonged shedding. Persons with more severe to critical illness or severe immunocompromise likely remain infectious no longer than 20 days after symptom onset, however. The CDC considers either a test- or stringent symptom-based strategy to be reasonable for those with low tolerance for postrecovery SARS-CoV-2 shedding and infectious risk. This group includes individuals who pose a risk of transmitting infection to vulnerable individuals at high risk for morbidity or mortality from SARS-CoV-2 infection or who support critical infrastructure. CDC has yet to recommend testing as part of healthcare worker (HCW) surveillance; in the absence of a significant known infection rate or the need to care without benefit of sufficient personal protective equipment (PPE), such a surveillance program is likely to be low-yield. If a testing-based strategy is to be used to assess individuals for whom there is low tolerance for viral shedding and infectious risk, a RTPCR test based upon an upper respiratory sample is probably adequate.

The utility of a diagnostic test is more directly dependent on positive and negative predictive value, which depends upon disease prevalence, rather than on the underlying sensitivity and specificity of the test itself. Predictive values can be readily calculated from sensitivity, specificity, and disease prevalence data using an online calculator (83). With low-prevalence disease, even a relatively poor test will give few false-negative results; a test with 50% sensitivity has a false negative rate only slightly over 5% when the prevalence is 10% (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/CCM/F737). When the actual prevalence of disease in the tested population is 20% or more as some laboratories have found during the pandemic, a test must have sensitivity upwards of 80% to achieve a similar false-negative rate. Although the best tests (both NAAT and serology) currently available have analytical sensitivity and specificity exceeding 99%, even low-levels of nonspecificity can have important implications. In the case of serologic testing, for which reports of past symptoms may not help to enrich the population for positive results, a population with a past infection prevalence of 5% and a test with 99% sensitivity and specificity give a false-positive rate of about 16%; in a population with 20% prevalence, the false-positive rate is less than 4%.

HEALTHCARE WORKER SURVEILLANCE AND PROTECTION

Although serologic studies cited earlier suggest that the community infection rate is higher than that confirmed by RTPCR testing, it is unclear whether this comes from asymptomatic transmission or failure to test minimally symptomatic individuals. Since both presymptomatic (84) and asymptomatic (85, 86) transmission may contribute to the spread of SARS-CoV-2 infection, some have called for testing healthcare workers (HCWs) to help prevent nosocomial spread (87). In Houston, about 5% of asymptomatic HCWs dealing directly with COVID19 patients were RTPCR positive—a rate considerably higher than that of non-COVID19 facing HCWs (88). Thirty-six percent of HCW in New York's Mount Sinai Healthcare System demonstrate an antibody response to SARS-CoV-2 (89). Similarly, 44% of workers and 23% of patients in the Indianapolis Riley’s Children's hospital were seropositive by May 5, 2020. Risk to HCW in skilled nursing facilities may be extremely high—in one facility, 64% of residents tested positive for SARS-CoV-2; 56% of them were asymptomatic at the time of testing (90). In contrast, one HCW screening program found infection rates that tracked closely with those of the surrounding community (91). CDC currently recommends that HCW be screened on the basis of symptoms rather than through a testing strategy (92). However, screening of asymptomatic patients and HCW may be inappropriate in some settings (93), particularly given high levels of exposure identified at some sites. This may help to reduce asymptomatic transmission that may lead to symptomatic disease in compromised individuals. Many, if not most, presymptomatic infections are associated with viral loads that are readily detected by any NAAT, including Abbott ID NOW (94). If a testing-based strategy is to be implemented, modeling studies suggest that routine testing should occur daily (95).

CONCLUSIONS AND RECOMMENDATIONS

Acquisition of a good specimen is critical for diagnosis of SARS-CoV-2 infection. RTPCR-based tests provide the greatest diagnostic sensitivity. LRT samples are more likely to yield a definitive diagnosis than URT specimens, but in most cases, a nasopharyngeal, mid turbinate, or nasal specimen is adequate. Patient self-sampling reduces PPE use and exposure of healthcare personnel, with diagnostic yield similar to that of clinician-obtained samples (Table 3, fourth data line) (28). Rapid NAAT tests may be useful for patient management, and in most populations perform well; the Cepheid Xpert Xpress system has a significantly higher sensitivity for SARS-CoV-2 than does the Abbott ID NOW system, but a longer turnaround time (~45 min instead of ~15 min). Serologic testing is most useful for population surveillance but should not, in general, be used to make an initial diagnosis of COVID19.
Alternative swabbing devices (including foam and spun polyester nasal swabs) and transport media have given results equivalent to those of nasopharyngeal swabs transported in VTM. VTM alternatives include MEM, PBS, and 0.9% saline. Dry swabs have also performed well. As shortages of sampling materials occur, clinicians should work with their laboratories to validate alternatives with their testing system.

There are no data to suggest that most patients need to be tested to assess the resolution of disease or discharge from isolation. The CDC recommends that, for patients with mild to moderate infections, isolation be maintained for at least 10 days “after illness onset” (the date symptoms begin) and at least 1 day (24 hr) “after recovery” (resolution of fever with no fever-reducing medication, together with progressive improvement/resolution of other symptoms) (12). Release from isolation and precautions should not occur for at least 20 days after onset of symptoms for those with severe illness (12).

Although recovery is accompanied by cessation of viral shedding (95), people who are immunocompromised by disease or medication may have shedding prolonged. The CDC considers either a test- or stringent symptom-based strategy to be reasonable for those with low tolerance for postrecovery SARS-CoV-2 shedding and infectious risk, and notes that persons with severe illness or immunocompromise likely remain infectious no longer than 20 days after symptom onset. This group includes individuals who pose a risk of transmitting infection to vulnerable individuals at high risk for morbidity or mortality from SARS-CoV-2 infection or who support critical infrastructure. CDC has yet to recommend testing as part of HCW surveillance; in the absence of a significant known infection rate or the need to care without benefit of sufficient PPE, such a surveillance program is likely to be low-yield. If a testing-based strategy is to be used to assess individuals for whom there is low tolerance for viral shedding and infectious risk, a RTPCR test based upon an upper respiratory sample is probably adequate.

The rate at which new information is appearing regarding both laboratory testing and clinical management of SARS-CoV-2 infection mandates an ongoing dialogue between patient-facing clinicians and laboratory clinicians. Communication can optimize diagnostic strategies, not only for the virus itself but for all testing needed to manage critically ill patients. Neither patient-facing clinicians nor laboratory clinicians have the bandwidth to adequately keep abreast with the rapid changes without the help of the other. Continuous mutual education engaging both clinic and laboratory personnel will assure optimal patient care while overcoming the limitations of this (or any) review article.

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