Deployment of COVID-19 diagnostic testing: challenges and key considerations for the future

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April 2, 2021

Introduction

Since the outbreak of COVID-19 there has been an unprecedented effort from the scientific community to develop tools to help tackle this crisis. According to the Foundation for Innovative New Diagnostics (FIND; 18 March 2021) there are 1025 commercialized SARS-CoV-2 assays currently available and an additional 98 in development, including 655 immunoassays and 437 molecular assays.[1] Early in the pandemic, molecular testing using nucleic acid amplification tests (NAATs) became the pillar of COVID-19 diagnostics. Since then, the development of antigen tests, immunoassays, point-of-care, and centralized options means there are now choices to be made as to how, when, and where to deploy these technologies and many guidelines have been developed, often based on Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) guidance.[2-4] However, with the rapid emergence of new technologies and new scientific data, these guidelines are very fluid and subject to change. Furthermore, while NAATs are the most sensitive diagnostic tool for COVID-19 infection,[3-5] the global demand for diagnostic testing is still such that the use of labour-intensive, specialist techniques needs to be carefully considered.

The aim of this review is to provide an international evaluation of real-world testing needs and to define: settings where the 'next best' alternatives to NAATs are appropriate; settings where NAATs may not be the best option; how to manage antigen test results; and how to manage negative NAAT results where there is still a strong clinical suspicion of COVID-19. We will further aim to set out the key considerations for defining a testing strategy. Table 1 demonstrates that each testing strategy provides different information on infection status and has different performance metrics, so the right option for the right setting needs to

be carefully assessed. Here we also discuss the common challenges facing clinicians and laboratorians when interpreting and supplying COVID-19 diagnostics and provide insights into what will be needed next.

Table 1. A summary of the diagnostic testing methodologies for COVID-19.

	Measure	Platforms/technologies	Turnaround time (range)	Number of samples per run/test	Performance range LOD sensitivity/specificity (%)
NAATs for viral RNA antigen detection (NP swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid, others)	Direct **detection of** SARS-CoV-2 viral RNA	High throughput RT-PCR[6]	1.5–8 hours	Up to 384	>1.23 cp/μL[7] 450–540,000 NDU/mL[8] >90%/up to 100%
		Point-of-care RT-PCR	20 mins	1	>12 cp/mL[7]
		High-throughput TMA	3 hours	Unconfirmed	600 NDU/mL[9]
		Point-of-care LAMP	20–60 mins	1	>10 cp/μl[7] >75% sensitivity[10]
		High-throughput LAMP (fluorescence)	45 mins	96	>1 cp/μl
		CRISPR/LAMP lateral flow	15 mins	1	>6.75 cp/μL[7]
Antigen detection (saliva, NP swab)	Immunoassays for **the detection of** SARS-CoV-2 viral antigens	High-throughput centralized	From 18 mins	Up to 300	Sensitivity (95% CI) <5 days post symptom onset and Ct <30: 97.5% (92.8–99.5%), Ct >30: 26.7% (12.3–45.9%)[11]

	Measure	Platforms/technologies	Turnaround times (range)	Number of samples per run/test	Performance range LOD sensitivity/specificity (%)
		Point-of-care (lateral flow)	15–30 mins	1	Sensitivity (95% CI): 28.9% (16.4–44.3) – 98.3% (91.1–99.7) Specificity (95% CI): 92.4% (87.4– 95.9) –100% (99.7–100)[12]
Antibody detection (serum, plasma)	**Detection of immune response** i.e. past exposure to SARS-CoV-2	High-throughput centralized	First results from 18 mins to 24 hours	Up to 500	Typically, >90% sensitive and >95% specific[13]
		Point-of-care (lateral flow)	15 mins	1	Typically, >90% sensitive and >95% specific[13]

CI, confidence interval; cp, copies; CRISPR, clustered regularly interspaced short palindromic repeats; Ct, cycle threshold; LAMP, isothermal loop-mediated amplification; NAAT, nucleic acid amplification test; NDU, NAAT detectable units; NP, nasal pharyngeal; RNA, ribonucleic acid; RT-PCR, reverse transcription polymerase chain reaction; TMA, transcription-mediated amplification.

1.1 Available classes of diagnostic tests

In September 2020, the WHO set out their target product profiles for COVID-19 diagnostics, stating that only NAATs are recommended for confirmation of COVID-19 disease.[14] Most NAATs have been based on reverse transcription polymerase chain reaction (RT-PCR), which enables highly sensitive and specific detection of viral RNA by targeting specific viral genes and amplifying the signal.[8,14,15] Transcription-mediated amplification (TMA) is another technique used interchangeably with RT-PCR that involves the isothermal amplification of RNA by reverse transcription and subsequent generation of numerous transcripts by RNA polymerase.[16] Loop-mediated isothermal amplification (LAMP) is a NAAT which utilizes an isothermal reaction that does not require the thermocycling process of RT-PCR.[17-19]Studies indicate that the LAMP technique is as highly specific as RT-PCR-based technologies but reports of sensitivities vary, with some studies reporting low sensitivity kits being marketed to developing countries.[19-22] However, LAMP can be performed with minimal equipment and has been deployed to supplement widescale testing and/or where resources are limited.[23]

In cases where NAATs are unavailable, turnaround times are unacceptably slow, or near-patient NAATs are necessary, rapid antigen tests may facilitate earlier diagnosis.[14,19] Antigen tests are typically immunoassays designed to detect SARS-CoV-2 proteins and require no amplification. As a result, these assays often require

less instrumentation and can be performed rapidly, often in near-patient settings rather than laboratories.[24,25] This class of tests may allow patients to self-sample and supports high-throughput testing.[24-27] However, antigen tests offer reduced sensitivity compared with NAATs, so adoption of these tests needs to be appropriate to the needs of the patient population served or the defined use-case (e.g. screening for same-day travel).[28]

Whilst NAATs are capable of diagnosing current infection, antibody testing identifies exposure to the pathogen over the patient's lifetime, supporting diagnosis later during the disease course.[29] Antibody testing aids our understanding of COVID-19 and our immune response,[30-33] the spread of infection,[14,34-36] and, more recently, our response to vaccine administration and long-term efficacy.[37]

Different testing settings

In this review, we will focus our discussion on 7 key testing settings: symptomatic individuals presenting for diagnostic testing and/or treatment of COVID-19 symptoms; asymptomatic individuals accessing healthcare for planned non-COVID-19-related reasons; patients needing to access emergency care (symptom status unknown); patients being discharged from healthcare following hospitalization for COVID-19; healthy individuals in both single event settings (e.g. airports, restaurants, hotels, concerts, sporting events) and repeat access settings (e.g. workplaces, schools, universities); and vaccinated individuals. These 7 settings comprise the key areas where testing is being used to care for patients and help to prevent the spread of infection, and echoes the list provided by the WHO.[3]

Before diagnostic testing is considered in any individual, it is important to establish if they have symptoms and, if so, the time from symptom onset.[2,4,5] On an individual basis, these simple factors will be key to determining the relevant test choice as, for example, antigen testing may not be beneficial >10 days post symptom onset.[38,39]

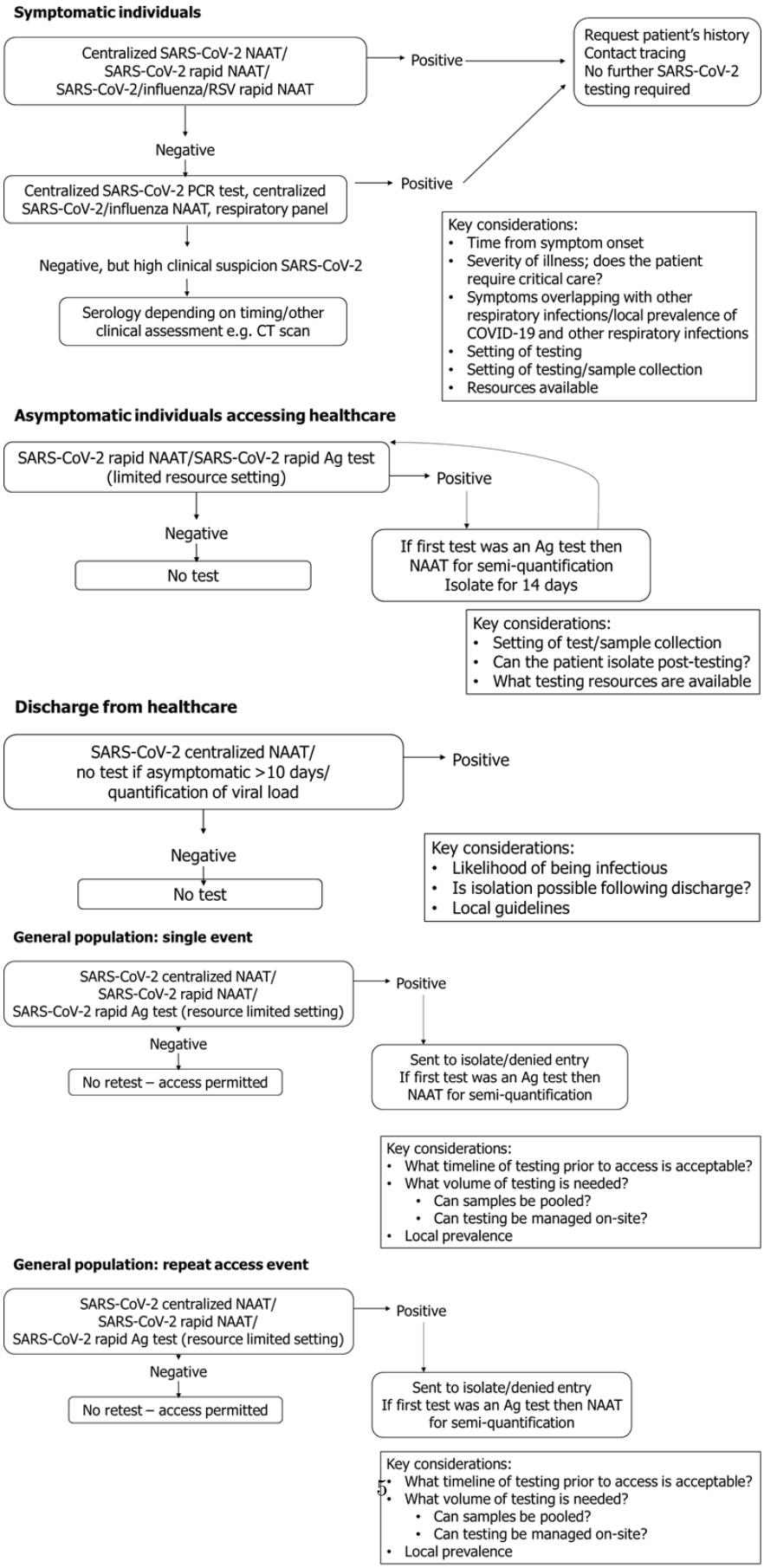

Figure 1. Testing strategies and considerations for the different diagnostic settings considered in this publication.

Ag, antigen; CT, computed tomography; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction.

Symptomatic patients

Current practices and guidelines/recommendations

The priority for symptomatic patients is the need to know if their symptoms are due to COVID-19; in these cases, NAATs for COVID-19 should be performed when possible (Figure 1). NAATs are the most sensitive class of tests available, and this method will help to ensure that cases are not missed among symptomatic patients.[26,40] In this context we are talking about RT-PCR testing and TMA, not LAMP, as sensitivity data are still variable (Table 1).[17-20,23,41-43] In settings where testing resources are available but limited, many laboratories have adopted sample pooling strategies that allow conservation of resources.[44]Several commercially available assays have regulatory authorization for pooling and offer guidance regarding the optimal number of samples to pool and the volume per sample to include in the pool.[45,46] The methods and benefits of pooling are highly influenced by the prevalence in the population being tested. For example, creating pools of 10 samples in a population of 10% positivity would require repeat testing of all of the individual samples in most pools, thus resulting in extra testing and an extended time to results. Conversely, pooling only 3 samples in a population with 1% positivity does not realize all of the reagent savings possible. Pooling strategies must be evaluated at each laboratory based on the population(s) they serve and can even be applied to sub-groups of samples sent to the laboratory to minimize time to results and maximize reagent conservation.[47-49]

In settings where NAATs are unavailable, antigen testing is also acceptable for the diagnosis of symptomatic patients as an option that is more informative than no testing. Antigen tests detect viral proteins in a patient's serum or plasma, and whilst they have a lower sensitivity than NAATs, they are most sensitive when viral loads are high, which may correlate with infectivity.[50] If symptoms are strongly indicative of COVID-19, a negative test should also be confirmed with a NAAT.[51-54] The authors consider that specificity is not an issue with currently available antigen tests, and that whilst retesting is not needed to confirm positivity, NAATs may be performed to provide semi-quantitative cycle threshold (Ct) values to aid understanding of infection status.[26,28,52,54-56] The utility of Ct values is currently unclear and the use of Ct values to assess infection status is currently only deployed in certain regions, and only then in patients who require medical intervention for COVID-19.

Depending on the local prevalence and patient-specific risk of influenza, dual-target NAATs for influenza and COVID-19 may be useful for differential diagnosis, particularly if an initial NAAT result is negative and clinical suspicion of respiratory infection is high (Figure 1). However, in many regions the prevalence of influenza is very low, possibly due to infection control measures for COVID-19, and the risk of influenza is lower than the normal risk expected for many regional flu seasons.[57-61]

If a patient repeatedly tests negative, but their clinical presentation is highly suggestive of COVID-19 and a diagnosis is required to enable medical care, a low-dose chest-computed tomography (CT) scan could be used to diagnose or rule out COVID-19.[62-64] However, this is recommended with caution, as chest-CT scans are less sensitive than NAATs for COVID-19, and specificity is often over-estimated due to selection bias and the low prevalence of other pulmonary disease in retrospective studies. The data suggest that chest-CT scans can be used to complement diagnostic testing but are not an effective standalone assessment.[62,63]

Key considerations

The key determinants of the test for use in symptomatic patients include the patient's symptoms/clinical presentation; whether the patient needs to be admitted for their symptoms or can manage their symptoms at home with isolation; and in the setting where patients are accessing testing/sampling and presenting to the healthcare system.[65] Globally, there are vast differences in how and where symptomatic individuals access healthcare, such as walk-in/fever clinics, drive-through testing centers, at-home testing squads, postal testing, and in the hospital/emergency department (ED)/general (not COVID-specific) clinic/COVID-specific clinic. If patients are accessing testing in a setting where they could possibly pass infection on to others, strict hygiene measures need to be applied and sample collection needs to be done as quickly as possible. If patients are well enough not to require urgent admission, then centralized testing is acceptable. However, if patients need urgent medical care for their symptoms, then rapid testing at the point of care is required. Several NAATs have been developed that can be performed in near-patient settings and, if available and affordable, these offer advantages over antigen-based assays. In symptomatic individuals, test sensitivity is important to ensure that infectious individuals are not missed and do not continue to spread their infection, whilst also ensuring that those who need medical care are appropriately triaged.

Asymptomatic hospital admissions

Patients in this category would need testing prior to hospital admission in order to prevent nosocomial COVID-19 transmission and to triage patients appropriately within the hospital setting.[66,67] These patients would not have symptoms of COVID-19 and would be attending routine healthcare appointments or planned surgeries; these appointments are not considered as urgent care.

Current practices

Point-of-care or centralized NAATs are generally used to test patients prior to admission. Patients may be able to isolate whilst they await their test results, making centralized testing possible in some settings. The urgency of the care that is required also determines whether the patient should have a rapid point-of-care test or whether a centralized option would be acceptable. As patients are asymptomatic there is no need for repeat testing; if they test positive, they should isolate for 14 days and then retest for COVID-19.[67] As these patients are asymptomatic, antigen tests may not be adequately sensitive to detect COVID-19 (Table 1).[4,53,68] In this setting, if patients do have a positive antigen test result they should be treated similarly to patients with a positive NAAT, requiring them to isolate for 14 days and retest (Figure 1).

Key considerations

NAATs are the most sensitive method for detection of SARS-CoV-2 and would maximally prevent the spread of infection to the healthcare system. A disadvantage of using NAATs in this setting is that studies have shown prolonged NAAT positivity in patients who are no longer symptomatic following infection, and it may be that non-infectious virus is detected.[50,69,70] [71]

Antigen testing is not as sensitive as a NAAT, particularly in asymptomatic individuals, and as such, there is the possibility that false-negative tests may leave the healthcare system exposed to infection.[72] However, this risk is currently mitigated by the universal COVID-19 precautions utilized in healthcare settings and will be further mitigated once the healthcare workforce is vaccinated. Antigen tests would be more useful if they could reliably indicate whether or not a patient is infectious, preventing unnecessary hospitalization. More information regarding how diagnostics tests relate to infectivity is needed before this is possible.

Urgent hospital admission, asymptomatic/symptom status unknown

Current practices

When patients need critical care, point-of-care NAATs will provide swift and accurate results (Figure 1). These patients are not being admitted due to symptoms of COVID-19 and, as such, repeat testing following a negative NAAT test would not be required, unless indicators arise to suggest a patient does have respiratory symptoms.

Key considerations

If results can be obtained more quickly using an antigen test in this clinical setting, then an antigen test in the interim is also acceptable; however, the result should be confirmed with a NAAT, which might be performed on site in a centralized laboratory.[66] In patients with respiratory symptoms in the ED setting, antigen testing has still been shown to produce false-negative results.[66] Depending on the clinical setting and the care that the patient requires, other assessments for the presenting condition may also reveal the likelihood of a respiratory infection but are not diagnostic for COVID-19.[73]

Discharge from healthcare following treatment for COVID-19

Current practices

Globally, the approach to managing patients leaving healthcare following COVID-19 is variable. In the US and Japan, patients are expected to be symptom free for a period of at least 10 days; in Germany, patients must have a Ct value <30 for discharge to nursing homes; in Italy, the absence of symptoms and a negative NAAT are the requirements for discharge.[74-76] Other countries only require a negative NAAT if the patient was severely unwell (e.g. receiving supportive oxygen).[74] In China, patients are discharged if they are no longer symptomatic and have a repeated negative NAAT for COVID-19 within 24 hours.[74] Chinese patients are then required to isolate for a further 14 days and may be discharged to an interim/recovery hospital for further isolation and monitoring before returning home.[70] These stringent criteria are due to reports of relapsing infection and aim to prevent transmissions in these cases.[77]

Key considerations

Patients are tested prior to discharge to ensure they are not infectious. Particular patient populations, such as post-transplant and/or immunocompromised patients, stay PCR-positive for a longer time period.[78] Ct values can provide a guide to infection status – a recovered patient with a high Ct corresponds with a low viral load, where a patient is unlikely to be infectious.[79,80] However, Ct values need to be standardized as there is variability between platforms, between labs, and between reagent lots within a single lab.[79] As such, they can provide clinical guidance only once the standardization practices for estimating viral concentration from Ct values is in place. Ct values are not routinely standardized against viral concentration ranges and, additionally, not all NAATs provide a Ct, meaning Ct values currently have limited clinical utility.[8079] In the future, quantification of viral load and standardization of Ct values may be widely applicable, aiding determination of infectious periods and possibly reducing the duration of hospitalization for some patient populations. In patients who remain NAAT-positive for a prolonged time period, antigen testing may better reflect if a patient is still infectious, as previously described above.

The general population

Single event

Here we define a 'single event' as an event that will not be routinely repeated with the same group of individuals (e.g. accessing an airport for travel, visiting a restaurant, or visiting a stadium as a spectator). This category encompasses a broad range of scenarios and recommendations will need to be specific for each setting.

Single event current practices

There is no standardized approach to testing within the community, both NAATs and antigen tests are being used. As an example scenario, many airlines and airports are developing pre-flight testing requirements, and currently these have not been regulated by the international community or even by local governments.[81]

When considering the appropriate test, if individuals can isolate between their test and the access event, then centralized NAATs could be used to deliver high volumes (e.g. people accessing an airport or a stadium event). In this asymptomatic population, pooling samples could be useful in order to maximize the testing capacity, although this may reduce the sensitivity of tests and, as such, is most appropriate for use with centralized PCR testing as the most sensitive method of detection.[44,47-49,82] However, this would not be practical for activities such as visiting a shopping center or restaurant. Highly sensitive antigen or NAAT point-of-care tests could be performed by non-laboratory trained personnel in these settings; however, whilst these tests provide quick results (10–15 minutes), they may not be suitable to conduct in crowded environments. Lateral flow antigen tests could be a simple and cost-effective way to test large groups of people; however, studies report high numbers of false-negative and false-positive results, meaning COVID-19 precautions should still be employed.[28,83,84]

Single event key considerations

When considering testing of the general asymptomatic population for access to single events, the key considerations should include:

- Population issues (e.g. if the population are asymptomatic). What is the local prevalence of infection? Are people attending from higher prevalence regions? This is very important as testing the general population may result in a high number of false-positive results[83]
- Sensitivity requirements for this population and the potential impact of a false-negative result. Is it necessary to detect all infected individuals, or only anyone highly infectious? Is catching the 'most infectious' cases sufficient? Will other measures, such as mask wearing and social distancing, be possible in this setting?
- The feasibility of testing at scale. What scale of testing is possible in that setting? Is reliable testing feasible? If self-swabs are used, how is the quality checked and will swabbing be supervised? What is the prevalence of infection?

Repeat access

Repeat access settings comprise workplaces, universities, schools, and hospitals, where the same group of people repeatedly interact together.

Repeat access current practices

Testing in repeat access settings is already being widely conducted, for example, many hospitals are regularly testing their healthcare staff using NAATs.[85] In healthcare staff, regular testing is leading to the identification

of many COVID-19 cases, enabling prompt isolation and therefore limiting outbreaks within hospitals.[86] In healthcare settings, testing staff has clear benefits for the costs involved with screening: the prevention of COVID-19 outbreaks among hospital staff, subsequent staff absences, and nosocomial transmission to potentially vulnerable patients. Pooling samples can also help to make screening these populations more resource efficient.[44,82]

Professional athletes and their support staff around the world have been subject to regular testing so that sports can continue during the pandemic. This is often supported by isolation, social distancing, personal protective equipment for staff, and other measures to prevent infection.[87-89] In these professional settings, the funding is often available to test regularly to ensure that the sports continue to operate, and these decisions sit with the sporting leagues and national governments.

For schools and universities, NAATs may not be needed, as antigen testing or LAMP may be sufficient to detect the most infectious cases. In addition, older students may be able to adhere to some social distancing and mask wearing measures. Overall, transmission has been noted to be lower in younger pupils compared with older pupils.[90,91] Whilst the cost–benefit of NAATs for screening in school students would likely prove inappropriate, antigen testing may be suitable. Pooling NAAT samples could also help to manage NAAT testing volumes; however, this should be approached cautiously as methods are not standardized and pooling techniques are not automated, which does compromise efficiency.[44]

In these repeat access settings, a single infection could become an outbreak. Importantly, local prevalence of infection and necessity of the contact should be carefully considered before allowing any gathering of individuals.

Repeat access key considerations

Similarly to single access events, the prevalence of infection is an important consideration when sampling is being carried out, as well as the frequency of testing. In Germany, healthcare workers are currently tested on a weekly basis using a NAAT; however, testing frequency will need to be determined on the basis of each situation, the risk of infection, and the cost–benefit.

Vaccinated individuals

Several COVID-19 vaccines have now been shown to provide protection against COVID-19 and many more are still under development.[92,93] There are still many unanswered questions regarding the longevity of immunity offered by vaccines, if they will be efficacious against all strains of COVID-19, which vaccines are most efficacious in different patient cohorts, and if vaccinated individuals are able to acquire and transmit COVID-19 without becoming infected. Studies are ongoing to answer all these questions using a range of testing strategies.

In order to assess the longevity of vaccine-mediated immunity, high-throughput quantitative anti-spike (S) antibody tests (as most vaccines currently elicit a response against the SARS-CoV-2 S- antigen) will be useful.[94] Anti-nucleocapsid antibody tests may also be used to assess natural versus vaccine-mediated immunity.[95-97] Many serological assays have been shown to correlate with neutralizing antibody titres;[98,99]however, direct assessment of neutralizing antibodies is preferable where possible as it is not fully understood how antibody test positivity relates to protective immunity against SARS-CoV-2.[96,100] Assessments of cellular immunity are also necessary to completely understand how COVID-19 vaccines offer protection and how long this protection lasts.

In order to assess whether new variants are emerging that have the potential to escape vaccine-mediated immunity, full genome analysis is needed to better track evolution and spread of lineages, with particular focus on regular S gene sequence analysis and vaccinated sera challenge studies of emergent strains.[101,102]

What are the testing considerations for the next steps in the pandemic?

A main focus for diagnostics will now be the ongoing monitoring of emergent strains. As discussed, this will be essential to ensure that the global rollout of vaccines is successful and to help the international community emerge from the pandemic.[101]Currently, as most NAATs detect several SARS-CoV-2 genetic targets, it is considered unlikely that mutations will lead to false-negative results; however, the FDA have requested that laboratorians are mindful that this may occur.[103]

Standardization of Ct values or fully standardized quantitative NAATs for SARS-CoV-2 will be extremely useful to assess efficacy of interventions in COVID-19 patients, to help determine when individuals are safe to leave quarantine and staff are safe to return to work.[104] Development of reference material to enable standardization of these assays is recommended.

The data regarding the relationship between diagnostic parameters and infectivity is currently limited, and research is ongoing in this area to clarify exactly how these parameters correlate. Recommendations on the most appropriate diagnostic test for different utilities may change once more information is available on these points.

The use of alternative sample types to the nasal pharyngeal (NP) swab, such as saliva, and the use of new technologies, such as CRISPR-based tests, are under investigation, and these may also provide different opportunities for testing and additional considerations (e.g. those concerning school populations or mass testing of large groups).[30,43,105,106] Self-sampling methods that provide increased sensitivity could broaden the capacity for mass testing prior to events or entering the workplace.[27]As it will take time before vaccination will be able to eradicate COVID-19, testing measures will continue to be important.

Acknowledgements

Medical writing support was provided by Rose-Marie Falconer at Elements Communications and was funded by Roche Diagnostics.

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