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Molecular accuracy vs antigenic speed: SARS-CoV-2 testing strategies

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Abstract

The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has hit every corner of the world faster than any infectious disease ever known. In this context, rapid and accurate testing of positive cases are essential to follow the test-trace-isolate strategy (TETRIS), which has proven to be a key approach to constrain viral spread. Here, we discuss how to interpret and combine molecular or/and antigen-based detection methods for SARS-CoV-2 as well as when they should be used. Their application can be cleverly designed as an algorithm to prevent viral dissemination according to distinct epidemiological contexts within surveillance programs.

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Introduction

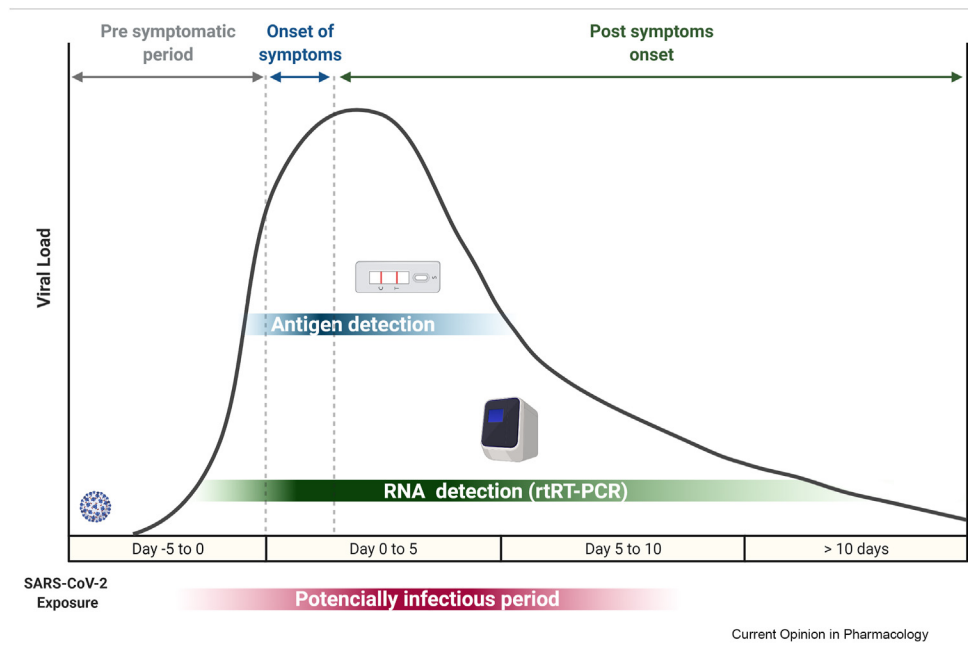
The rapid and accurate identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a matter of priority since the first cases of infection by this virus, that causes the Coronavirus Disease 2019 (COVID-19), were detected in late December 2019 [1]. Since then, different approaches have been exploited in order to develop reliable diagnostic tools which are essential to follow the TETRIS

(i.e., test, trace and isolate) program [2]. This strategy consists of the detection and quarantine of positive individuals, followed by the trace of their contacts, who are asked to isolate until tested. This approach has shown to be successful in controlling viral spread, preventing health care systems saturation, and reducing the economic impact of isolation measures [3,4]. However, the selection of the most convenient detection techniques for the diagnosis will depend on the goals pursued according to the testing strategy. Broadly speaking, different reliable methods can be distinguished, each with different strengths and limitations according to the target population and the turn-around time. Herein, we focus on the most widely available diagnostic tests, and consequently, the most suitable for diagnostic and screening purposes.

When sensitivity matters: nucleic acid detection

Real-time reverse transcription-polymerase chain reaction (rtRT-PCR) is the most commonly used diagnostic method for detecting viral infections. It is considered the gold standard technique to detect SARS-CoV-2 positive cases due to its high specificity and high analytical sensitivity (or low detection limit) [5,6]. By definition, the detection limit is the lowest analytical signal that can be detected in more than 95% of the replicates performed. Therefore, the lower this value, the greater the chance of detecting infected individuals who may be contagious despite being asymptomatic or at early or late stages of the infection period, where viral loads are low (Figure 1). An increasing number of rtRT-PCR assays have been approved for diagnostic use under emergency authorization by the U.S. Food and Drug Administration (FDA) [7] and the European Commission (CE) [8]. These strategies are designed to specifically detect different targets of SARS-CoV-2 genome, mainly structural proteins genes, such as spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as open reading frames 1a or 1b. Most of these assays simultaneously target two or three viral genome regions to reduce false-negative PCR results which may occur as a consequence of viral mutations [9]. It should be noted that other molecular-based diagnostic methods like droplet digital PCR (ddPCR) [10], Reverse

Figure 1



SARS-CoV-2 infection time course and detection windows according to antigen and rtRT-PCR tests. The viral load and the diagnostic tests results represented in the scheme are based on nasopharyngeal swab samples. The time period indicated here refers to an unscaled range due to the infectious course diversity observed in SARS-CoV-2 positive cases. The onset of symptoms is stated at Day 0 and can result in different viral loads as represented.

Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) [11], and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based techniques [12] have been increasingly developed, and although they have high specificity, their sensibility is generally below rtRT-PCR assays and their availability is still limited [13].

Despite the high reliability of rtRT-PCR, certain characteristics undermine its use on a large scale. Although sample processing may take a couple of hours, the logistics of sample collection, transportation, processing, analysis, and results delivery, can generate bottlenecks delaying the identification and isolation of positive cases. Other drawbacks are its cost, and the need for specialized laboratories with specific equipment and trained personnel [14].

In addition, due to its high analytical sensitivity, rtRT-PCR can be over-sensitive and detect individuals who are in the final phase of the infection when they are no longer contagious [15] (Figure 1). This is due to viral genetic traces that can remain for long periods in the clinical sample, in particular sub-genomic fragments that encode for structural proteins, which are synthesized at high levels during SARS-CoV-2 replicative cycle [16]. These viral mRNAs are suggested to be generated in cytoplasmic double-membrane vesicles during

transcription and replication stages and have been observed to persist tightly associated with them for up to 22 days after the onset of symptoms, providing nuclease protection [15,17]. Consequently, rtRT-PCR positivity does not necessarily indicate the infective capacity of the individual. In fact, with exceptions [18,19], replication-competent viruses cannot be recovered 10 days after symptom onset in patients with mild to moderate COVID-19 [20–24]. In the latter studies, infectivity assays were carried out as a proxy to assess the potential contagiousness of infected individuals. Unfortunately, these strategies are incompatible to implement in diagnostic schemes due to their complexity, the requirement for biosafety level 3 (BSL-3) facilities, and the time required for their analysis.

Antigenic tests, “the fastest gun”

Under widespread community transmission, the increase in suspected cases of SARS-CoV-2 infections generates the need to apply screening tests that allow extensive surveillance. Antigen tests are immunoassays that detect the presence of a specific viral antigen, which indicates current viral infection. These tests, also referred to as rapid tests, offer multiple advantages, including low costs, short turn-around times, and ease of processing [25]. Since no laboratories or highly trained personnel are required for its implementation, it can be used at point-of-care and patient self-testing.

Most antigenic tests have been developed by immobilizing monoclonal antibodies against SARS-CoV-2 antigens (mainly found in nucleoprotein (N) or spike (S) proteins) in lateral flow devices [26]. These immunoassays have proven to be highly accurate to detect symptomatic individuals during the peak of infection (Figure 1) [27,28]. Nevertheless, they are generally less sensitive than rtRT-PCR and other nucleic acid amplification approaches. Antigenic tests are commonly used to monitor personnel in at-risk environments such as hospitals, schools, entertainment centers, and for extensive screening where a new COVID-19 outbreak is suspected [29]. Since May 2020, an increasing number of SARS-CoV-2 antigen tests have been approved by the FDA and the CE for diagnostic use under emergency authorization [7,8].

Antigen tests generally yield legible results in less than 30 min and can be performed onsite without any sample processing or specific instrument requirements. Low viral load restricts sensitivity and could be responsible for false negative results [30]. A positive result depends on performing the test at the precise moment of the course of the infection as the persistence and stability of the viral antigens is limited (Figure 1).

Which tests should be used and in what scenarios?

The application of objective-driven testing strategies for SARS-CoV-2 significantly supports the public health response to the pandemic and contributes to mitigate its impact on vulnerable people and healthcare structures. Consequently, it ensures that economies and societies can keep working whereas the following aspects are pursued: i) controlling transmission rates and severity cases; ii) diminishing the impact of COVID-19 in healthcare and social care facilities; iii) identifying clusters or outbreaks in certain locations; and iv) keeping SARS-CoV-2 transmission under control.

The clinical performance of diagnostic tests largely depends on the specimen type, time of collection, and the circumstances in which they are used. Currently, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) recommend the collection of upper respiratory tract samples for the diagnosis of SARS-CoV-2 [31,32]. General procedures for sampling collection should be followed as the accuracy of the test largely depends on pre-analytic variables related to the specimen quality and the sampling timing during the acute phase of the disease [13,33]. Nasopharyngeal swabs are considered the highest yield specimens for these purposes [34]. Nasal and oropharyngeal swabs, as well as saliva, have been suggested as suitable alternatives [35], although further studies are needed to evaluate their performances [36–38]. In COVID-19 hospitalized patients with lower respiratory

tract diseases, endotracheal aspirates, bronchoalveolar lavage fluid, and sputum may be considered and have even shown greater persistence of the SARS-CoV-2 viral load compared to samples from the upper respiratory tract [39,40].

The median incubation period (the time elapsed from the infection with SARS-CoV-2 and the onset of symptoms) is around 5–7 days [41–44]. This period corresponds to the time when viral load peaks, both for symptomatic and asymptomatic individuals (Figure 1) [45,46]. Therefore, both molecular and antigenic tests will have a better performance with sampling times at the beginning of symptoms or around day 5–7 after infection (Figure 1).

Testing strategies should be dynamic and adaptable to evolving epidemiological scenarios. Thus, detection methods of choice must rely on several factors that should be specifically weighed in order to implement effective approaches to constrain the dissemination of SARS-CoV-2. On the basis of the CDC metrics, the level of community transmission can be considered low when the percentage of positive SARS-CoV-2 diagnostic nucleic acid amplification tests in the last 7 days is below 5% [47]. In a community with low viral circulation, priority should be given to detect the largest number of positive cases, including patients with low viral loads who may be asymptomatic or pre-symptomatic. Several studies have evidenced culturable viruses in patients with significantly low viral loads [20,48,49]. Moreover, asymptomatic and pre-symptomatic transmissions have been extensively reported [50–54]. Thus, in these epidemiological contexts, highly sensitive detection methods as molecular-based techniques must be used to quarantine positive individuals before they increase their contagiousness. By these means, false-negative results are limited, which may arise when using low-sensitivity diagnostic tools. In addition, as long as the prevalence remains low, sample-pooling strategies can be effectively applied to increase the number of samples and reduce costs, especially in resource-limited situations [55]. It should be noted that, as stated before, although infectious viruses are rarely recovered in viral culture after 10 days of symptoms, prolonged viral RNA shedding has been evidenced in upper respiratory tract samples up to 12 weeks post-onset of symptoms [18,56,57]. Consequently, we must emphasize that a positive result by rtRT-PCR should not be considered as an indicator of active infection, but should be associated with available data about the onset and duration of symptoms to evaluate the likelihood of transmission of the infected individual.

A different scenario occurs in high prevalence settings where the use of highly sensitive diagnostic techniques

becomes a second order of priority, prevailing the strategies that allow large populations screening in short turn-around times [14]. In these epidemiological contexts, the focus should be put on the rapid detection and isolation of people with high viral loads, as they may act as super spreaders [58,59]. Highly contagious individuals have proven to shape the course of the pandemic, being the major drivers of viral transmission [58,59]. Consequently, under these circumstances, antigen-based methods should be implemented as they can reliably detect symptomatic individuals when they are more likely to disseminate the virus (typically during the first week of illness) (Figures 1 and 2) [44]. Therefore, rapid diagnostic tools should play a pivotal role in large-scale surveillance programs, strategically combined with molecular detection assays for optimal patient management (Figure 2).

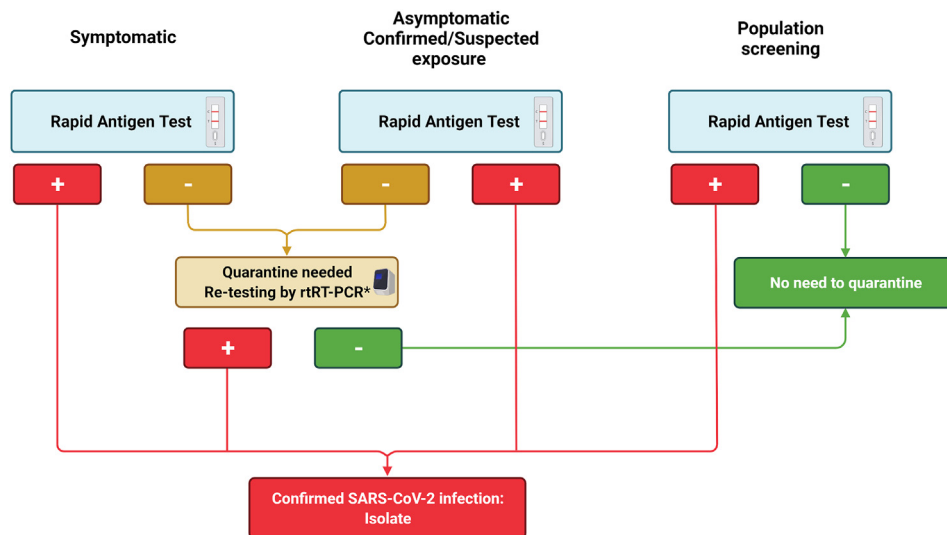
It is worth mentioning that post-infection and post-vaccination immunity confers strong protection against severe COVID-19, but subsequent infections can still occur [60,61]. Due to waning immunity and emergence of viral variants of concern, the risk of reinfection/breakthrough infection increases with time after recovery/vaccination [62–68]. However, viral loads, infectious virus shedding, and symptoms tend to be significantly reduced both in magnitude and duration, which limits the risk of onward transmission [69–71]. It is therefore important to evaluate diagnostic test results

on the basis of clinical context of the individual being tested including infection/vaccination status, presence or absence of symptoms and close contact with COVID-19 patients. A negative antigen result may need to be confirmed by an rtRT-PCR test when the person is symptomatic or asymptomatic with a recent confirmed or suspected contact with a positive case (Figure 2). Also, antigen testing is very useful to allow safe return to normal activities or to attend recreational or social events. In this context, antigen tests are used to mitigate the risk and a negative result does not need a confirmatory test. In the case of positive individuals, the interruption of isolation measures should be based mainly on the resolution of the symptoms, rather than test-based. Epidemiological data have shown that quarantine can be discontinued 10 days after the onset of symptoms, or 10 days after the positive test in asymptomatic cases [72].

Concluding remarks

Molecular-based diagnostic methods, especially rtRT-PCR, remain the most reliable tools for the detection of active SARS-CoV-2 infections. However, the lower costs, simplicity, and possibility for point-of-care use turn rapid antigenic tests into a powerful alternative, particularly in high community transmission contexts, as well as a tool to allow safe return to normal activities. Public health strategies can take advantage of the pros and cons

Figure 2



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Suggested algorithm for the implementation of SARS-CoV-2 antigen tests and requirements to couple with rtRT-PCR tests for an accurate diagnosis. Population Screening refers to the test performed to asymptomatic individuals with no known contact with a confirmed/suspected SARS-CoV-2 positive case, in order to attend recreational or social events, school screening, and international traveling in a vaccinated context. In this group, the antigen negative result may not need confirmatory testing due to low likelihood of SARS-CoV-2 infection. * In the case of a negative antigen test result, quarantine should be performed until a new nasopharyngeal swab sample is obtained (5–7 days post the onset of symptoms or post confirmed/suspected exposure with a SARS-CoV-2 positive case) and processed by a rtRT-PCR assay.

of both technologies and complementarity use them to tackle the challenge of limiting viral dissemination.

Conflict of interest statement

Nothing declared.

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