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

Álvaro Fajardo[1,2,a], Paula Perbolianachis[1,2,a], Irene Ferreiro[1,2], Pilar Moreno[1,2] and Gonzalo Moratorio[1,2]

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.

Addresses

[1] Laboratorio de Evolución Experimental de Virus, Institut Pasteur, Montevideo, Uruguay
[2] Laboratorio de Virología Molecular, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

Corresponding authors: Moratorio, Gonzalo (moratorio@pasteur.edu.uy); Moreno, Pilar (pmoreno@fcien.edu.uy)
[a] Contributed equally.

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 analytic 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
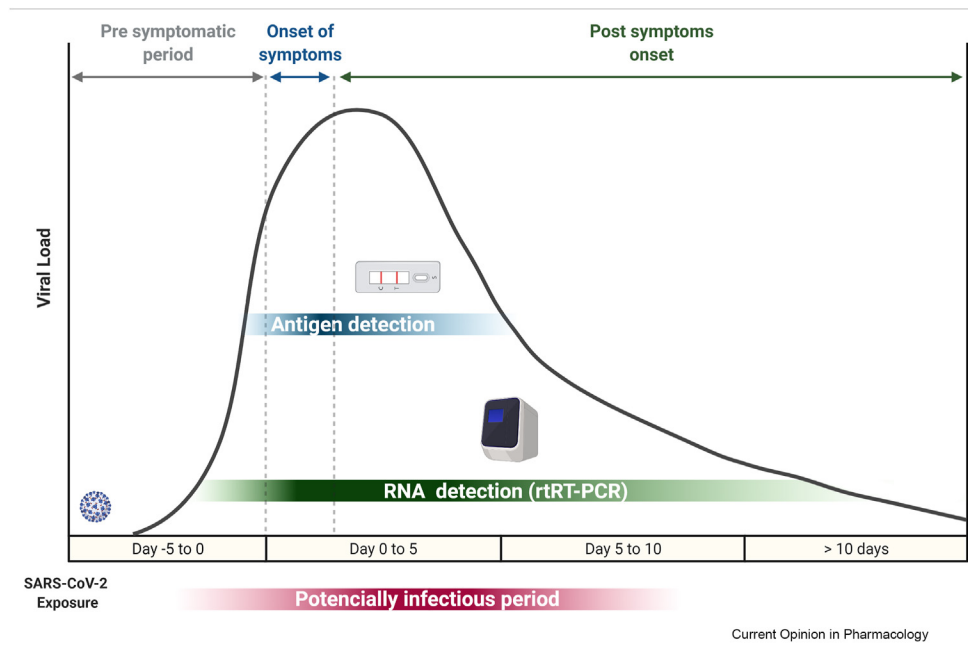
SARS-CoV-2 infection time course and detection windows according to antigen and rtRT-PCR tests. The viral load and the diagnostic tests results represented in the scheme are based on nasopharyngeal swab samples. The time period indicated here refers to an unscaled range due to the infectious course diversity 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 presymptomatic. Several studies have evidenced culturable viruses in patients with significantly low viral loads [20,48,49]. Moreover, asymptomatic and presymptomatic 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
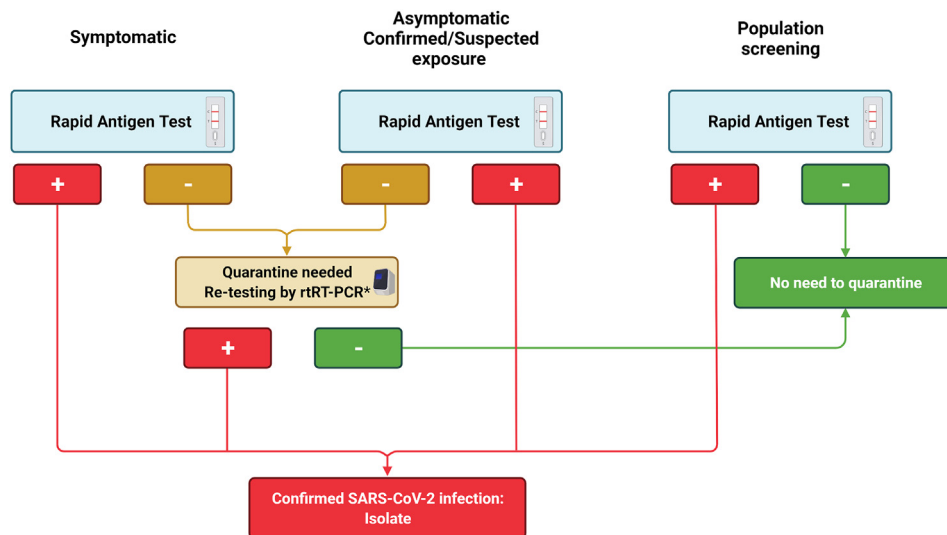
Current Opinion in Pharmacology

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.

References
Papers of particular interest, published within the period of review, have been highlighted as:

* * of outstanding interest

1. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, Hu Y, Tao Z-
* * W, Tian J-H, Pei Y-Y, et al.: **A new coronavirus associated with human respiratory disease in China**. *Nature* 2020, **579**: 265−269.
Seminal paper of the discovery of SARS-CoV-2

2. Contreras S, Dehning J, Loidolt M, Zierenberg J, Spitzner FP, Urrea-Quintero JH, Mohr SB, Wilczek M, Wibral M, Priesemann V: **The challenges of containing SARS-CoV-2 via test-trace-and-isolate**. *Nat Commun* 2021, **12**:1−13.

3. Moreno P, Moratorio G, Iraola G, Fajardo Á, Aldunate F, Pereira-Gómez M, Perbolianachis P, Costábile A, López-Tort F, Simón D, et al.: **An effective COVID-19 response in South America: the Uruguayan Conundrum**. *medRxiv* 2020, https://doi.org/10.1101/2020.07.24.20161802.

4. Chung S-C, Marlow S, Tobias N, Alogna A, Alogna I, You S-L, Khunti K, McKee M, Michie S, Pillay D: **Original research: lessons from countries implementing find, test, trace, isolation and support policies in the rapid response of the COVID-19 pandemic: a systematic review**. *BMJ Open* 2021, **11**.

5. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, Xu H: **Positive RT-PCR test results in patients recovered from COVID-19**. *JAMA* 2020, **323**:1502−1503.

6. Arnaout R, Lee RA, Lee GR, Callahan C, Cheng A, Yen CF, Smith KP, Arora R, Kirby JE: **The limit of detection matters: the case for benchmarking severe acute respiratory syndrome coronavirus 2 testing**. *Clin Infect Dis* 2021, https://doi.org/10.1093/CID/CIAA1382.

7. *In vitro diagnostics EUAs*. FDA; 2021. Available online: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas. Accessed 7 October 2021.

8. *COVID-19 in vitro diagnostic devices and test methods database*. 2021. Available online: https://covid-19-diagnostics.jrc.ec.europa.eu/. Accessed 7 October 2021.

9. Tahan S, Parikh BA, Droit L, Wallace MA, Burnham CAD, Wang D: **Sars-cov-2 e gene variant alters analytical sensitivity characteristics of viral detection using a commercial reverse transcription-pcr assay**. *J Clin Microbiol* 2021, **59**.

10. Liu C, Shi Q, Peng M, Lu R, Li H, Cai Y, Chen J, Xu J, Shen B: **Evaluation of droplet digital PCR for quantification of SARS-CoV-2 Virus in discharged COVID-19 patients**. *Aging (Albany NY)* 2020, **12**:20997−21003.

11. Yu L, Wu S, Hao X, Dong X, Mao L, Pelechano V, Chen W-H, Yin X: **Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform**. *Clin Chem* 2020, **66**: 975−977.

12. Hou T, Zeng W, Yang M, Chen W, Ren L, Ai J, Wu J, Liao Y, Gou X, Li Y, et al.: **Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19**. *PLoS Pathog* 2020, **16**, e1008705.

13. Abduljalil JM: **Laboratory diagnosis of SARS-CoV-2: available**
* * **approaches and limitations**. *New Microbes New Infect* 2020, **36**: 100713.
This study shows that patients with severe COVID-19 may shed replication-competent virus for longer periods than patients with mild COVID-19. They also observe the absence of infectious viruses when the patient exhibit low viral loads and serum neutralizing antibodies, suggesting the utility of combining quantitative RNA assays and serological methods to infer the likelihood of infectiousness.

14. Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, Hay JA, Tambe M, Mina MJ, Parker R: **Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening**. *Sci Adv* 2021, **7**.

15. van Kampen JJA, van de Vijver DAMC, Fraaij PLA,
* * Haagmans BL, Lamers MM, Okba N, van den Akker JPC, Endeman H, Gommers DAMPJ, Cornelissen JJ, et al.: **Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19)**. *Nat Commun* 2021, **12**:1−6.
The authors evidence the prolonged detection of SARS-CoV-2 subgenomic RNAs in samples obtained up to 17 days after the positive diagnosis. Considering that viral replication and transcription occurs in double-membrane vesicles, and that the resulting complexes can persist nuclease resistant for long periods, they suggest that RT-PCR results alone cannot indicate the infectiousness of the patient.

16. Kim D, Lee J-Y, Yang J-S, Kim JW, Kim VN, Chang H: **The architecture of SARS-CoV-2 transcriptome**. *Cell* 2020, **181**: 914−921. e10.

17. Alexandersen S, Chamings A, Bhatta TR: **SARS-CoV-2 genomic and subgenomic RNAs in diagnostic samples are not an indicator of active replication**. *Nat Commun* 2020, **11**:1−13.

18. Liu W-D, Chang S-Y, Wang J-T, Tsai M-J, Hung C-C, Hsu C-L, Chang S-C: **Prolonged virus shedding even after seroconversion in a patient with COVID-19**. *J Infect* 2020, **81**:318−356.

19. Tarhini H, Recoing A, Bridier-nahmias A, Rahi M, Lambert C, Martres P, Lucet J-C, Rioux C, Bouzid D, Lebourgeois S, et al.: **Long-term severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectiousness among three immunocompromised patients: from prolonged viral shedding to SARS-CoV-2 superinfection**. *J Infect Dis* 2021, **223**:1522−1527.

20. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, et al.: **Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples**. *Clin Infect Dis* 2020, **71**:2663−2666.

21. Lu J, Peng J, Xiong Q, Liu Z, Lin H, Tan X, Kang M, Yuan R, Zeng L, Zhou P, et al.: **Clinical, immunological and virological characterization of COVID-19 patients that test re-positive for SARS-CoV-2 by RT-PCR**. *EBioMedicine* 2020, **59**:102960.

22. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, et al.: **Virological assessment of hospitalized patients with COVID-2019**. *Nature* 2020, **581**:465−469.

23. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng O-T, Marimuthu K, Ang LW, Mak TM, et al.: **Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore**. *JAMA* 2020, **323**:1488−1494.

24. Owusu D, Pomeroy MA, Lewis NM, Wadhwa A, Yousaf AR, Whitaker B, Dietrich E, Hall AJ, Chu V, Thornburg N, et al.: **Persistent SARS-CoV-2 RNA shedding without evidence of infectiousness: a cohort study of individuals with COVID-19**. *J Infect Dis* 2021:1−10.

25. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z: **Considerations for diagnostic COVID-19 tests**. *Nat Rev Microbiol* 2020, **19**:171−183.

26. Fabiani L, Caratelli V, Fiore L, Scognamiglio V, Antonacci A,
* * Fillo S, Santis R De, Monte A, Bortone M, Moscone D, et al.: **State of the art on the SARS-CoV-2 toolkit for antigen detection: one year later**. *Biosens* 2021, **11**:310.
The authors analyzed 251 upper respiratory samples obtained during the first week of symptoms of COVID-19 patients. They observe that

antigenic tests showed a higher positive prediction value than RT-PCR (90% vs 70%) when compared to the presence of replication-competent viruses. Therefore, they suggest that antigen tests can be more reliable than RT-PCR tests in assessing the infectiousness of the individual when samples are obtained during the acute phase of the infection.

27. Mina MJ, Andersen KG: **COVID-19 testing: one size does not fit all**. *Science (80-)* 2021, **371**:126−127.

28. Pekosz A, Parvu V, Li M, Andrews JC, Manabe YC, Kodsi S, Gary DS, Roger-Dalbert C, Leitch J, Cooper CK: **Antigen-based testing but not real-time polymerase chain reaction correlates with severe acute respiratory syndrome coronavirus 2 viral culture**. *Clin Infect Dis* 2021, https://doi.org/10.1093/CID/CIAA1706.

29. *Overview of testing for SARS-CoV-2 (COVID-19)*. CDC; 2020. Available online: https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html. Accessed 7 October 2021.

30. Guglielmi G: **Fast coronavirus tests: what they can and can't do**. *Nature* 2020, **585**:496−498.

31. *Interim guidelines for clinical specimens for COVID-19*. CDC; 2020. Available online: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. Accessed 7 October 2021.

32. Boodman C, Lagacé-Wiens P, Bullard J: **Diagnostic testing for SARS-CoV-2**. *CMAJ (Can Med Assoc J)* 2020, **192**:E1608.

33. Rabaan AA, Tirupathi R, Sule AA, Aldali J, Mutair A Al, Alhumaid S, Muzaheed Gupta N, Koritala T, Adhikari R, *et al.*: **Viral dynamics and real-time RT-PCR Ct values correlation with disease severity in COVID-19**. *Diagnostics* 2021, **11**:1091.

34. Lee RA, Herigon JC, Benedetti A, Pollock NR, Denkinger CM: **Performance of Saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: a systematic review and meta-analysis**. *J Clin Microbiol* 2021, **59**.

35. Safiabadi Tali SH, LeBlanc JJ, Sadiq Z, Oyewunmi OD, Camargo C, Nikpour B, Armanfard N, Sagan SM, Jahanshahi-Anbuhi S: **Tools and techniques for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/COVID-19 detection**. *Clin Microbiol Rev* 2021, **34**.

36. Congrave-Wilson Z, Lee Y, Jumarang J, Perez S, Bender JM, Bard JD, Pannaraj PS: **Change in Saliva RT-PCR sensitivity over the course of SARS-CoV-2 infection**. *JAMA* 2021, **326**:1065−1067.

37. Nacher M, Mergeay-Fabre M, Blanchet D, Benoit O, Pozl T, Mesphoule P, Sainte-Rose V, Vialette V, Toulet B, Moua A, *et al.*: **Prospective comparison of saliva and nasopharyngeal swab sampling for mass screening for COVID-19**. *Front Med* 2021: 176.

38. Riccò M, Ranzieri S, Peruzzi S, Valente M, Marchesi F, Balzarini F, Bragazzi NL, Signorelli C: **RT-qPCR assays based on saliva rather than on nasopharyngeal swabs are possible but should be interpreted with caution: results from a systematic review and meta-analysis**. *Acta Biomed Atenei Parm* 2020, **91**, e2020025.

39. Tang YW, Schmitz JE, Persing DH, Stratton CW: **Laboratory diagnosis of COVID-19: current issues and challenges**. *J Clin Microbiol* 2020, **58**.

40. Winichakoon P, Chaiwarith R, Liwsrisakun C, Salee P, Goonn A, Limsukon A, Kaewpoowat Q: **Negative nasopharyngeal and oropharyngeal swabs do not rule out COVID-19**. *J Clin Microbiol* 2020, **58**.

41. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, Liu L, Shan H, Lei C,
** Hui DSC, *et al.*: **Clinical characteristics of coronavirus disease 2019 in China**. *N Engl J Med* 2020, **382**:1708−1720, https://doi.org/10.1056/NEJMoa2002032.
The authors analyzed data (demographic characteristics, exposure history, and illness timelines) of the first 425 confirmed COVID-19 cases in Wuhan. Different epidemiologic characteristics of this disease were determined, including the mean incubation period (5.2 days) and the basic reproductive number (2.2).

42. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, Azman AS, Reich NG, Lessler J: **The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application**. *Ann Intern Med* 2020, **172**:577−582, https://doi.org/10.7326/M20-0504.

43. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, *et al.*: **Early transmission dynamics in Wuhan, China, of novel coronavirus−infected pneumonia**. *N Engl J Med* 2020, **382**:1199−1207, https://doi.org/10.1056/NEJMoa2001316.

44. Zaki N, Mohamed EA: **The estimations of the COVID-19 incubation period: a scoping reviews of the literature**. *J Infect Public Health* 2021, **14**:638−646.

45. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, *et al.*: **Temporal dynamics in viral shedding and transmissibility of COVID-19**. *Nat Med* 2020, **26**:672−675.

46. Kissler SM, Fauver JR, Mack C, Olesen SW, Tai C, Shiue KY, Kalinich CC, Jednak S, Ott IM, Vogels CBF, *et al.*: **Viral dynamics of acute SARS-CoV-2 infection and applications to diagnostic and public health strategies**. *PLoS Biol* 2021, **19**, e3001333.

47. Christie A, Brooks JT, Hicks LA, Sauber-Schatz EK, Yoder JS, Honein MA: **Guidance for implementing COVID-19 prevention strategies in the context of varying community transmission levels and vaccination coverage**. *MMWR Morb Mortal Wkly Rep* 2021, **70**:1044−1047.

48. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, Gautret P, Raoult D: **Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards**. *Eur J Clin Microbiol Infect Dis* 2020, **39**:1059−1061.

49. Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, Ladhani S, Zambon M, Gopal R: **Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020**. *Euro Surveill* 2020, **25**:2001483.

50. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, *et al.*: **SARS-CoV-2 viral load in upper respiratory specimens of infected patients**. *N Engl J Med* 2020, **382**:1177−1179, https://doi.org/10.1056/NEJMc2001737.

51. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, *et al.*: **Transmission of 2019-nCoV infection from an asymptomatic contact in Germany**. *N Engl J Med* 2020, **382**:970−971, https://doi.org/10.1056/NEJMc2001468.

52. Qian G, Yang N, Ma AHY, Wang L, Li G, Chen X, Chen X: **COVID-19 transmission within a family cluster by presymptomatic carriers in China**. *Clin Infect Dis* 2020, **71**:861−862.

53. Kimball A: **Asymptomatic and presymptomatic SARS-CoV-2 infections in residents of a long-term care skilled nursing facility — King County, Washington, March 2020**. *MMWR Morb Mortal Wkly Rep* 2020, **69**:377−381.

54. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, Shaman J: **Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2)**. *Science (80-)* 2020, **368**:489−493.

55. Sawicki R, Korona-Glowniak I, Boguszewska A, Stec A, Polz-Dacewicz M: **Sample pooling as a strategy for community monitoring for SARS-CoV-2**. *Sci Rep* 2021, **11**:1−8.

56. Plebani M: **Persistent viral RNA shedding in COVID-19: caution, not fear**. *EBioMedicine* 2021, **64**:103234.

57. Li N, Wang X, Lv T: **Prolonged SARS-CoV-2 RNA shedding: not a rare phenomenon**. *J Med Virol* 2020, **92**:2286−2287.

58. Adam DC, Wu P, Wong JY, Lau EHY, Tsang TK, Cauchemez S, Leung GM, Cowling BJ: **Clustering and superspreading potential of SARS-CoV-2 infections in Hong Kong**. *Nat Med* 2020, **26**:1714−1719.

59. Lemieux JE, Siddle KJ, Shaw BM, Loreth C, Schaffner SF, Gladden-Young A, Adams G, Fink T, Tomkins-Tinch CH, Krasilnikova LA, et al.: **Phylogenetic analysis of SARS-CoV-2 in Boston highlights the impact of superspreading events**. *Science (80-)* 2021, **371**.

60. Cohen JI, Burbelo PD: **Reinfection with SARS-CoV-2: implications for vaccines**. *Clin Infect Dis* 2020, https://doi.org/10.1093/CID/CIAA1866.

61. Team CC-19 VBCI, Team CC-19 VBCI, Team CC-19 VBCI, Birhane M, Bressler S, Chang G, Clark T, Dorough L, Fischer M, Watkins LF, et al.: **COVID-19 vaccine breakthrough infections reported to CDC — United States, January 1–April 30, 2021**. *Morb Mortal Wkly Rep* 2021, **70**:792.

62. Zucman N, Uhel F, Descamps D, Roux D, Ricard J-D: **Severe reinfection with South African severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant 501Y.V2**. *Clin Infect Dis* 2021, https://doi.org/10.1093/CID/CIAB129.

63. Harrington D, Kele B, Pereira S, Couto-Parada X, Riddell A, Forbes S, Dobbie H, Cutino-Moguel T: **Confirmed reinfection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant VOC-202012/01**. *Clin Infect Dis* 2021, https://doi.org/10.1093/CID/CIAB014.

64. Nonaka CKV, Franco MM, Gräf T, de L Barcia CA, de Á Mendonça RN, de Sousa KAF, Neiva LMC, Fosenca V, Mendes AVA, de Aguiar RS, et al.: **Genomic evidence of SARS-CoV-2 reinfection involving E484K spike mutation, Brazil - volume 27, number 5—May 2021 - emerging infectious diseases journal - CDC**. *Emerg Infect Dis* 2021, **27**: 1522–1524.

65. Resende PC, Bezerra JF, Vasconcelos RHT, Arantes I,
** Appolinario L, Mendonça AC, Paixao AC, Duarte AC, Silva T, Rocha AS, et al.: **Severe acute respiratory syndrome coronavirus 2 P.2 lineage associated with reinfection case, Brazil, June–October 2020**. *Emerg Infect Dis* 2021, **27**:1789.
This study indicate that BNT162b2 vaccine prevents COVID-19 for up to 6 months, despite the emergence of SARS-CoV-2 variants.

66. Naaber P, Tserel L, Kangro K, Sepp E, Jürjenson V, Adamson A, Haljasmägi L, Rumm AP, Maruste R, Kärner J, et al.: **Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study**. *Lancet Reg Heal - Eur* 2021, https://doi.org/10.1016/J.LANEPE.2021.100208.

67. Thomas SJ, Edson D, Moreira J, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Marc GP, Polack FP, Zerbini C, et al.: **Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine through 6 months**. *N Engl J Med* 2021, https://doi.org/10.1056/NEJMoa2110345.

68. Dolgin E: **COVID vaccine immunity is waning — how much does that matter?** *Nature* 2021, **597**:606–607.

69. Levine-Tiefenbrun M, Yelin I, Katz R, Herzel E, Golan Z,
** Schreiber L, Wolf T, Nadler V, Ben-Tov A, Kuint J, et al.: **Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine**. *Nat Med* 2021, **27**:790–792.
This study indicates the impact of vaccination in reducing the duration of symptoms and high transmission potential.

70. Thompson MG, Burgess JL, Naleway AL, Tyner H, Yoon SK, Meece J, Olsho LEW, Caban-Martinez AJ, Fowlkes AL, Lutrick K, et al.: **Prevention and attenuation of covid-19 with the BNT162b2 and mRNA-1273 vaccines**. *medRxiv* 2021, **385**: 320–329, https://doi.org/10.1056/NEJMoa2107058.

71. Ke R, Martinez PP, Smith RL, Gibson LL, Achenbach CJ, McFall S, Qi C, Jacob J, Dembele E, Bundy C, et al.: **Longitudinal analysis of SARS-CoV-2 vaccine breakthrough infections reveal limited infectious virus shedding and restricted tissue distribution**. *medRxiv* 2021, https://doi.org/10.1101/2021.08.30.21262701.

72. *Ending isolation and precautions for people with COVID-19: interim guidance*. 2021. Available online: https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html. Accessed 8 October 2021.