ORIGINAL ARTICLE

Evaluation of a rapid SARS COV-2 antigen detection test in comparison with real time RT-PCR assay in Tunisia

Évaluation d'un test antigénique rapide de détection du SARS COV-2 en comparaison avec la RT-PCR en temps réel en Tunisie

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Résumé

Introduction

En réponse à la pandémie croissante de COVID-19, de nombreux fabricants ont développé et commencé à vendre des tests de diagnostic rapide afin de gérer la crise de santé publique. L'objectif de cette étude était d'évaluer la performance du test antigénique rapide de détection du SARS COV-2 avec technique immunochromatographique «STANDARD Q COVID-19 Ag Test» (SD BIOSENSOR) en comparaison avec la méthode de référence, la PCR qualitative en temps réel par transcription inverse (rRT-PCR).

Méthodes

L'étude a porté sur 200 échantillons nasopharyngés congelés provenant de différents patients, préalablement testés pour le SARS-CoV-2 selon un protocole rRT-PCR développé par un laboratoire et basé sur le protocole de Hong Kong, au Centre National de la Grippe au laboratoire de microbiologie de l'hôpital Charles Nicolle. Les échantillons ont été divisés en deux lots : un premier lot de 100 échantillons avec des résultats négatifs de la rRT-PCR et un second lot de 100 échantillons avec des résultats positifs de la rRT-PCR, qui a été divisé en 4 catégories de 25 chacune selon la charge virale (très élevée, élevée, moyenne et faible). Tous les échantillons ont été testés avec le test antigénique rapide «STANDARD Q COVID-19 Ag Test» conformément aux recommandations du fabricant. Les résultats obtenus ont été comparés à ceux obtenus par la rRT-PCR.

Résultats

Les résultats des échantillons négatifs et des échantillons très fortement chargés étaient conformes à la méthode de référence. Dix-neuf des 25 échantillons fortement chargés étaient positifs. Cependant, pour les échantillons moyennement et faiblement chargés, 23/25 et 24/25 résultats faux négatifs ont été trouvés, respectivement. Les paramètres de performance ont montré une sensibilité de 47% et une spécificité de 100%.

Conclusion

L'avantage du test STANDARD Q COVID-19 Ag est qu'un résultat positif fiable peut être obtenu en 30 minutes chez les patients hautement suspects. Mais il est insuffisant pour exclure une véritable infection en cas de résultat négatif, ce qui nécessite une rRT-PCR.

Les mots clés: *SARS COV-2, COVID-19, Test antigénique, Test rapide d'orientation diagnostic*

Abstract

Introduction

In response to the growing COVID-19 pandemic, many manufacturers have developed and began selling Point Of Care tests in order to manage the current public health crisis. The aim of this study was to evaluate the performance of the SARS-COV-2 rapid antigen detection test with immunochromatography technique "STANDARD Q COVID-19 Ag Test" (SD BIOSENSOR) in comparison with the reference method, the qualitative real-time reverse transcription PCR (rRT-PCR).

Methods

The study included 200 frozen nasopharyngeal specimens from different patients, previously tested for SARS-CoV-2 using a laboratory-developed RT-qPCR assay based on the Hong Kong protocol, at the National Influenza Center at the Microbiology laboratory of Charles Nicolle Hospital. Specimens were divided into two sets: a first set of 100 samples with negative rRT-PCR results and a second set of 100 samples with positive rRT-PCR results, which was divided into 4 categories of 25 each according to viral load (very high, high, medium and low). All samples were tested with the SARS-COV-2 rapid antigen detection test "STANDARD Q COVID-19 Ag Test according to the manufacturer's recommendations. Results obtained were compared with those by the rRT-PCR.

Results

Negative samples results and very highly loaded samples results were consistent with the reference method. Nineteen out of 25 highly loaded samples were positive. However, for the medium and low loaded samples, 23/25 and 24/25 false negative results were found, respectively. Performance parameters showed a sensitivity of 47% and a specificity of 100%.

Conclusion: The advantage of the STANDARD Q COVID-19 Ag Test is that a reliable positive result can be obtained within 30 minutes in highly suspicious patients. But it is insufficient to rule out true infection in case of a negative result, which requires rRT-PCR**.**

Key words: *SARS COV-2, COVID-19, Antigenic Test, Point-of-Care Testing*

INTRODUCTION

In December 2019, a previously unknown betacoronavirus was discovered in samples from patients with pneumonia in Wuhan, China. This novel coronavirus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is the seventh member of the family of coronaviruses that infect humans(1). SARS-CoV-2 causes asymptomatic and mild diseases more than severe pneumonia and severe cases may develop acute respiratory distress syndrome (ARDS) and death (2). Globally, there have been over 150 million confirmed cases of COVID-19, including more than 4 000 000 deaths, reported to WHO (3). In Tunisia, the first confirmed case of coronavirus disease 2019 (COVID-19) was detected on the 2nd of March 2020 (4). As of 11 October 2021, there have been 710 096 confirmed cases with 25 046 deaths in our country (5).

The real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay remains the current standard test for laboratory diagnosis of SARS-CoV-2 infection. In many countries, access to this form of testing has been challenging (6). In addition, the laboratory diagnosis results by these assays are time-consuming and requires operations performed by skilled technicians (7). In response to the growing pandemic, manufacturers have developed Point of Care Tests (POCT) in order to manage the current public health crisis. Knowledge of diagnostic tests for SARS-CoV-2 is still evolving and a clear understanding of the nature of the tests and interpretation of their findings is important (8). Standard Q COVID-19 Ag test (SD Biosensor®, Chuncheongbuk-do, Republic of Korea) is a rapid CE-marked lateral flow chromatographic immunoassay for the detection of SARS-CoV-2 nucleocapsid (N) antigen in respiratory specimens (9, 10). It was accepted by the WHO Emergency Use Assessment COVID-19 IVDs within the framework of the EUL process (11). As a commitment to listing, the manufacturer was required to provide the real time stability studies report by 31 July 2022 (12).

Here we evaluated the diagnostic performances (specificity, sensitivity) of the rapid detection test "STANDARD Q COVID-19 Ag Test", in comparison with the reference method, the qualitative rRT-PCR.

METHODS

Specimen collection

Two hundred aliquots of nasopharyngeal specimens, collected between February and May 2020 with known SARS-CoV-2 infection status, and frozen at -80°C were used. The specimens were collected from patients meeting the Tunisian suspect patient definition: Symptomatic persons suspected of having SARS-CoV-2 infection with clinical signs suggestive of Covid-19 without other etiologies explaining the symptomatology, persons who have been exposed to a confirmed Covid-19 positive case or hospitalized for unexplained acute respiratory distress, clustered cases of acute respiratory infections even in the absence of travel or contact with a travel or contact with a confirmed case of Covid-19, asymptomatic or pauci-symptomatic health care workers who have had exposure to a confirmed Covid-19 case without adequate protection) (13), and travelers screened at a port of entry and in quarantine places. Aliquots were divided into two sets: A first set of 100 samples with a negative rRT-PCR result and a second set of 100 samples with a positive rRT-PCR result.

Viral RNA Extraction

Viral RNA was extracted using the QIAamp viral RNA QIAcube kits and the QIAGEN QIAcube automate. Extraction was performed according to the manufacturer's instructions.

SARS-CoV-2 RNA detection using real-time RT-PCR

PCR was performed using a laboratory-developed rRT-PCR assay according to the Hong Kong protocol based on two monoplex assays: the N gene RT-PCR as a screening assay and the Orf1b assay as a confirmatory one (14).

The 200 specimens included in the study were divided according to their RT-qPCR results into two groups: 100 negative and 100 positive specimens. The 100 RT-qPCR positive results were distributed into 4 sub-groupsof 25 each, according to viral load: Very high (ct value ∈ [14-20]), high (ct value ∈ [21-25]), medium (ct value ∈ [26-34]) and low (ct value ∈ [35-42]).

"STANDARD Q COVID-19 Ag Test"

STANDARD Q COVID-19 Ag has two precoated lines on the result window: Control (C) and test (T) lines on the surface of the nitrocellulose membrane. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region and mouse monoclonal anti-chicken Igγ antibody is coated on the control line region. Mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles allow the detection of SARS-CoV-2 antigen presented in the specimen. The antigen–antibody color particle complex migrates via capillary force until the test line where it will be captured by the mouse monoclonal anti-SARS-CoV-2 antibody (15). The test was performed using three drops of viral transport medium, in biosafety level-2 enhanced (BSL-2+) facilities with full personal protective equipment. Results were manually read after 30 minutes. Table I details the characteristics of the antigenic test.

Statistical analysis

Sensitivity was calculated as the proportion of true positive results detected by STANDARD Q COVID-19 Ag among all positives by the reference method (%).

Specificity was calculated as the proportion of true negative specimens, identified as negative by STANDARD Q COVID-19 Ag among all negatives by the reference method (%).

RESULTS

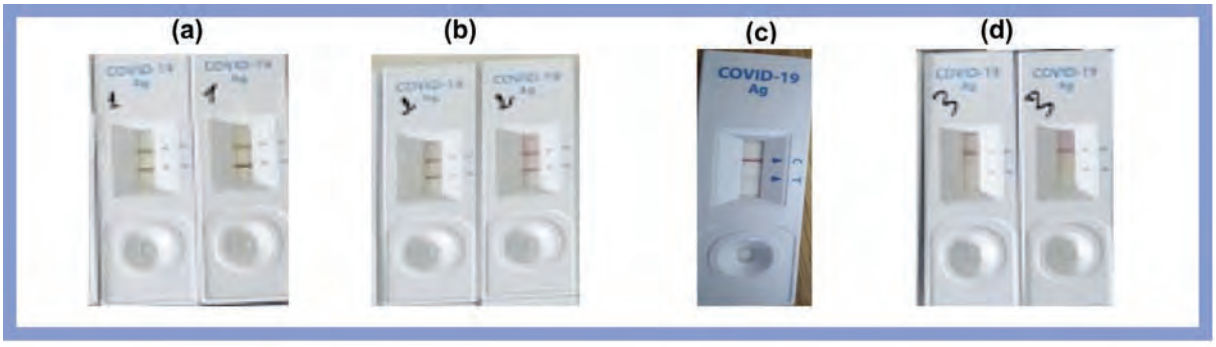
Using the STANDARD Q COVID-19 Ag test, the results were interpreted as positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within

Table I: Characteristics of the antigenic test

Test name	STANDARD Q COVID-19 Ag Test
Reference	Q-NCOV-01G
Lot Number	QCO302011
Pack size	25 tests per kit
Product Storage (temperature range)	2-30 °C
Shelf-life (months)	24 months
Manufacturing Site (country)	Republic of Korea
Specimen	Nasopharyngealswab
Target	Nucleocapsid
Process	Manual
Technological	Immunochromatographic
Procedure	Reading within 30 minutes. Positive if both
	control and test line are present. Negative if only the control band is Present. The colored test (T) line's intensity depends on the amount of SARS-CoV-2 antigen presented in the sample.

30 minutes. The colored test (T) line's intensity depends on the amount of SARS-CoV-2 antigen presented in the sample (figure 1). The results of each group of positive specimens are represented in table II. No invalid result was detected. All negative samples on RT-qPCR were negative using the STANDARD Q COVID-19 Ag test, supporting the excellent specificity of the test (100%). On the other hand, 47 out of 100 positive samples on RT-qPCR

Figure 1: Interpretation of Standard Q COVID-19 Ag Test.



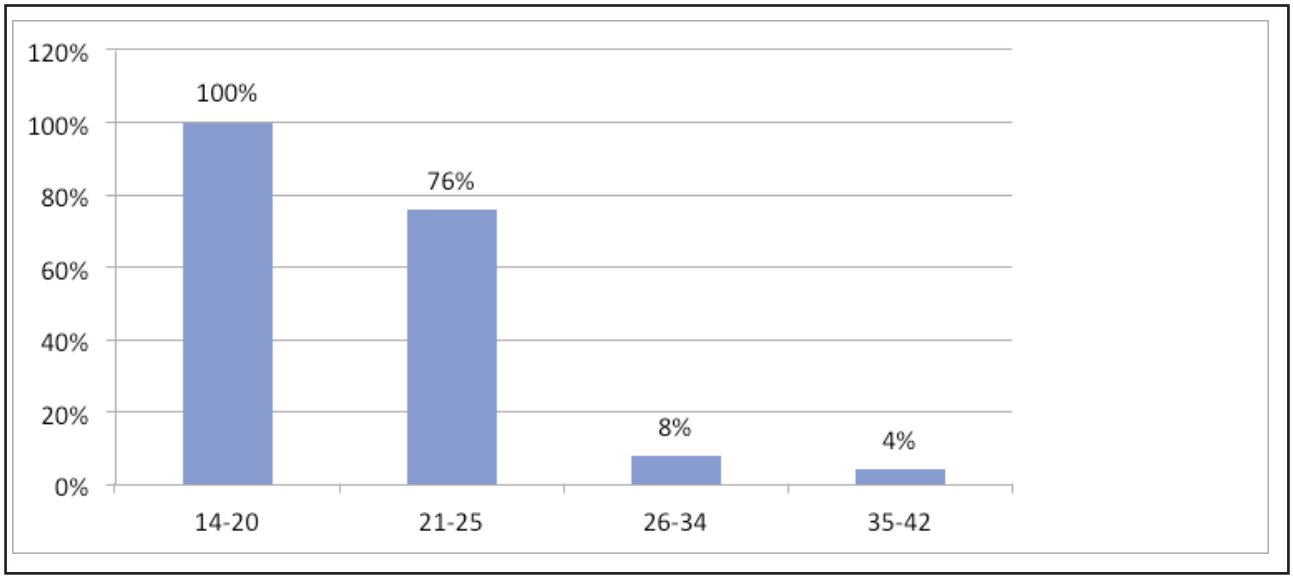
Demonstration of (a), (b)test strips interpreted as positive SARS-CoV-2 antigen, (c) test strip interpreted as weakly positive SARS-CoV-2 antigen and (d) test strips interpreted as negative SARS-CoV-2 antigen.

Table II: Results of the rapid antigen detection kit for the presence of SARS-CoV-2 virus in 100 positive respiratory specimens by RT-qPCR.

Specimen type	Ct value Range	No. of specimens positive by RT-qPCR	No. of specimens positive by the STANDARD Q COVID-19 Ag test
Very high viral load	14-20	25	25
High viral load	21-25	25	19
Medium viral load	26-34	25	2
Low viral load	35-42	25	1
All	14-42	100	47

have been tested positive using STANDARD Q COVID-19Ag Test. Very highly loaded samples results were consistent with the reference method; a total of 19 out of 25 highly loaded samples were positive. For the medium and low loaded samples, 23/25 and 24/25 false negative results were found, respectively. Although the global sensitivity of the rapid assay was suboptimal (47%), a satisfying sensibility when Ct ≤ 25 (88%) was noted. Figure 2 shows the sensitivity according to the ct value.

Figure 2: Sensitivity of the SARS-CoV-2 rapid test according to the Ct value



DISCUSSION

In this study, we determined the performance characteristics of the Standard Q COVID-19 Ag test for detecting SARS-CoV-2 virus. The overall sensitivity and specificity were 47% and 100% respectively. When Ct ≤ 34 (Opinion of 25/09/2020 of the French Society of Microbiology: «If the Ct value is ≤ 33, the presence of detected viral RNA is compatible with significant viral shedding») (16), sensitivity was 61%. For viral loads ≤ 25 Ct, i.e. high viral loads corresponding to the most contagious patients (17, 18), ensitivity was satisfying

(88%). However, manufacturer performance data shows a sensibility of 85% and a specificity of 98.4% (15). Performance characteristics presented by the manufacturer are based on a diagnostic evaluation conducted by the Foundation for Innovative New Diagnostics (FIND) (15, 19). In the latest version of FIND assessment, a prospective evaluation study of the test with a total number of 2192 enrolled individuals was conducted in Germany, Brazil and Switzerland. The clinical sensitivity was 90.5% when Ct ≤ 33 and 97.7% when Ct ≤ 25, while specificity was 98.9% (19). But it should be noted that the FIND study included patients with clinical symptoms of SARS-CoV-2 infection according to the claim of intended use from SD Biosensor, Inc. Also, variation of method to quantify viral load is noted. In the current study, the group «low viral loaded» samples included specimens with Ct values between 35 and 42, higher values than the Ct used in other evaluations. Furthermore, the test was conducted on fresh nasopharyngeal swabs according to the manufacturer's recommendation. A systemic review showed that STANDARD Q COVID-19 Ag was the best-performing test among 17 POCT for diagnosis of SARS-CoV-2 infection. Its overall sensitivity and specificity were 79.3% (95% CI 69.6% to 86.6%) and 98.5% (95% CI 97.9% to 98.9%). They were 80.1% (95% CI 68.5% to 88.1%) and 98.1% (95% CI 97.4% to 98.6%) in symptomatic patients. While they were 61.1% (95% CI 37.9% to 80.2%) and 99.6% (95% CI 97.3% to 99.9%) in asymptomatic people. Restricting to instructions for use (IFU)-compliant evaluations, average sensitivities and specificities were higher: 85.8% (95% CI 80.5% to 89.8%) and 99.2% (95% CI 98.2% to 99.6%) overall; 88.1% (95% CI 84.2% to 91.1%) and 99.1% (95% CI 97.8% to 99.6%) in symptomatic people; and 69.2% (95% CI 38.6% to 90.9%) and 99.1% (95% CI 95.2% to100%) in asymptomatic people (20). Thus, the test met the WHO acceptable criteria (i.e. sensitivity ≥ 80% and specificity ≥ 97%) based on pooled results of several studies (21). POCT are rapid, cost-efficient and easy to use (22-24). They could be a game changer in the COVID-19 pandemic by increasing access to testing and early confirmation of cases, particularly in low-resource settings (25). Moreover, the use of POCTs in mass screening testing could decrease the burden on laboratories and the shortage of reagent they are facing (10, 25). However, the lack of sensitivity compared to PCR assays is due to the fact that they do not involve an amplification step (26). Indeed, the review of HAS notes that the efficiency of viral antigen detection appears to be correlated with viral load (27). Therefore, false negative results will miss infectious people and result in outbreaks in countries that have largely controlled coronavirus transmission. Others view the lower sensitivity as an attribute, since some people with positive PCR test results are infected, but are no longer able to spread the virus to others. Thus, antigen tests could shift the focus to identifying the most infectious people (22). Cerutti *et al.,* reported that the majority of discordant RT-PCR-positive/Ag test-negative samples reported negative results when cell-cultured. False negative results were found in samples with a low viral load, consistent with low viable virus and low infectiousness (10). Rapid antigen tests are only sufficiently sensitive in the first week since onset of symptoms (20). According to HAS, antigenic tests are not recommended as surveillance tools in conditions of low virus circulation (27).

The main limit of this study is the retrospective evaluation using frozen specimens stored at -80°C in a viral transport medium and not on fresh nasopharyngeal swabs as recommended by the manufacturers. Freezing-thawing steps, time to freeze and the use of viral transport medium from an initial volume (the amount of antigen tested could be lower than that available from fresh specimens) may influence the performance of the assay (17). In addition, this was a laboratory-based study that did not provide details regarding symptom status. The monitoring of the performance in real conditions of use is essential. Therefore, a prospective study on fresh samples with information on symptoms and time from symptom onset should be performed.

CONCLUSION

Understanding the performance of POCT allows its adequate implementation. The advantage of the STANDARD Q COVID-19 Ag Test is that a reliable positive result can be obtained within 30 minutes in highly suspicious patients with high viral load in the upper respiratory tract, as rapid results are required for rapid patient care. In addition, it can spot those who are at greatest risk of spreading the disease. But it is insufficient to rule out true infection in case of a negative result, which requires an rRT-PCR. Tunisia has approved the use of this antigen test and it was rolled out in vast numbers of symptomatic patients for mass-screening for COVID-19.

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STANDARD Q COVID-19 SD-Biosensor kit was acquired in May 2020 by the laboratory from the Unit of Medical Biology Laboratories of the Ministry of Health, in the framework of the evaluation of this new medical device.

RÉFÉRENCES BIBLIOGRAPHIQUES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 20 févr 2020;382(8):727‑733.

2. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, *et al.* World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surg Lond Engl. avr 2020;76:71‑76.

3.WHO Coronavirus (COVID-19) Dashboard [Internet]. https://covid19.who.int

4. Chakroun H, Ben Lasfar N, Fall S, Maha A, El Moussi A, Abid S, *et al.* First case of imported and confirmed COVID-19 in Tunisia. Tunis Med. 2020; 98(4):258‑260.

5.ONMNE Tunisie | Ministère de la santé. https://onmne.tn/

6. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. https://www.who.int/publications-detail-redirect/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays

7. Choi JR. Development of Point-of-Care Biosensors for COVID-19. Front Chem. 2020; 8:517.

8. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 2020; 323(22):2249-2251.

9. Chaimayo C, Kaewnaphan B, Tanlieng N, Athipanyasilp N, Sirijatuphat R, Chayakulkeeree M, *et al.* Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. Virol J. 2020;17(1):177.

10. Cerutti F, Burdino E, Milia MG, Allice T, Gregori G, Bruzzone B,*et al.* Urgent need of rapid tests for SARS CoV-2 antigen detection: Evaluation of the SD-Biosensor antigen test for SARS-CoV-2. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2020;132:104654.

11. WHO Emergency Use Listing for In vitro diagnostics (IVDs) Detecting SARS-CoV-2. https://www.who.int/publications/m/item/200922-eul-sars-cov2-product-list

12. Coronavirus disease (COVID-19) Pandemic — Emergency Use Listing Procedure (EUL) open for IVDs. WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control). https://extranet.who.int/pqweb/vitro-diagnostics/coronavirus-disease-covid-19-pandemic-emergency-use-listing-procedure-eul-open

13. Guide parcours du patient suspect ou confirmé Covid-19 | INEAS. https://www.ineas.tn/fr/ actualite /guide-parcours-du-patient-suspect-ou-confirme-covid-19

14. The University of Hong Kong (HKU), School of Public Health. Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR. https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1 20.pdf

15. Products - STANDARD Q COVID-19 Ag. http://sdbiosensor.com/xe/product/7672

16. Covid-19 Fiches et Documents SFM. Société Française de Microbiologie. https://www.sfm-microbiologie.org/covid-19-fiches-et-documents-sfm/

17.Évaluation_de_la_performance_diagnostique_de_9 tests_rapides_antigéniques_(TRA)_COVID-19. calameo.com. https://www.calameo.com/ read/0040218274 bc351179414

18. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, *et al.* Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. Clin Infect Dis Off Publ Infect Dis Soc Am. 2020;71(10):2663-2666.

19. Home - FIND. https://www.finddx.org/

20. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, *et al.* Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2021; 3:CD013705.

21. COVID-19 Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0 . https://www.who.int/publications/m/item/covid-19-target-product-profiles-for-priority-diagnostics-to-support-response-to-the-covid-19-pandemic-v.0.1

22. Guglielmi G. Fast coronavirus tests: what they can and can't do. Nature. 16 sept 2020;585(7826):496-8.

23. Nguyen T, Duong Bang D, Wolff A. 2019 Novel Coronavirus Disease (COVID-19): Paving the Road for Rapid Detection and Point-of-Care Diagnostics. Micromachines. 2020;11(3):306.

24. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. Nat Rev Microbiol. mars 2021;19(3):171‑83.

25. Krüger LJ, Gaeddert M, Köppel L, Brümmer LE, Gottschalk C, Miranda IB, *et al.* Evaluation of the accuracy, ease of use and limit of detection of novel, rapid, antigen-detecting point-of-care diagnostics for SARS-CoV-2. medRxiv. 4 oct 2020; 2020.10.01.20203836.

26. Yüce M, Filiztekin E, Özkaya KG. COVID-19 diagnosis -A review of current methods. Biosens Bioelectron. 2021; 172:112752.

27. Revue rapide sur les tests de détection antigénique du virus SARS-CoV-2 Haute Autorité de Santé. https://www.has-sante.fr/jcms/p_3213483/fr/revue-rapide-sur-les-tests-de-detection-antigenique-du-virus-sars-cov-2.