Real-time PCR-based SARS-CoV-2 detection

José Francisco Cancino Mesa[1]* https://orcid.org/0000-0002-9328-9856
Adrián Alejandro Vitón Castillo[2] https://orcid.org/0000-0002-7811-2470
Jorge Casí Torres[3] https://orcid.org/0000-0003-2176-5187

[1] Granma University of Medical Sciences, Faculty of Medicine Celia Sánchez Manduley. Cuba
[2] Pinar del Río University of Medical Sciences, Faculty of Medicine Ernesto Guevara de la Serna. Pinar del Río. Cuba
[3] Clinical Surgical Hospital Celia Sánchez Manduley, Department of Internal Medicine. Manzanillo. Granma. Cuba.

Correspondence to: jcancinomesa@gmail.com

ABSTRACT

The reverse transcriptase-polymerase chain reaction is a high precision technique to detect and amplify genetic material.
Aim: To describe the use of the reverse transcriptase-polymerase chain reaction as a laboratory test for the detection of SARS-CoV-2.
Method: a literature review was conducted on articles published up to May 2020. A survey on the databases Scopus, Wiley Online Library, SciELO, DIALNET, EBSCO, MEDLINE and PubMed retrieved 40 articles of interest included in this research.
Development: the reverse transcriptase-polymerase chain reaction to detect SARS-CoV-2 targets the RNA polymerase dependent RNA, ORF1ab fragments, the E gene, the N gene and the S gene. Nasopharyngeal swabs offer better results than oropharyngeal swabs and saliva. The inclusion of reverse transcriptase-polymerase chain reaction tests (RT-PCR) using rectal swab in suspected false-negative cases is necessary. New studies and techniques are continuously developed to optimise the detection process.
Conclusions: the availability of diagnostic tests is crucial for the identification of positive cases and the traceability of the transmission chain. RT-PCR is the test of choice during the active period of viral replication. The RT-LAMP assay is a rapid diagnostic alternative with similar principles to RT-PCR.

Keywords: Polymerase Chain Reaction; Coronavirus Infections; Clinical Laboratory Techniques

INTRODUCTION

Coronaviruses belong to the subfamily Coronavirinae in the family Coronaviridae of the order Nidovirales(1). They are large, positive-sense RNA viruses comprising four genera: alpha, beta, delta and gamma(2). Until 2019, only six human coronaviruses (HCoV) were knew to be responsible for respiratory diseases. Two of them, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), are viral strains capable of infecting the lower respiratory tract(2).

In 2002, an outbreak of severe acute respiratory syndrome (SARS), caused by SARS-CoV, threatened the lives of 8,098 people with a mortality of 774 patients. The disease's epicentre was Guangdong, China; and it spread internationally to more than a dozen countries. Bats were assumed to be the natural hosts(3,4).

In 2012 the Middle East Respiratory Syndrome (MERS) caused by MERS-CoV emerged in Saudi Arabia. Bats became the natural hosts and the intermediate hosts were dromedary camels. A total of 2494 cases with 858 deaths, were reported by rapid nosocomial transmission. The MERS exhibited clinical features similar to SARS with prominent gastrointestinal symptoms and acute renal failure(3,4).

In December 2019, the Chinese Government alerted the international scientific community of a sporadic outbreak of pneumonia cases with no known underlying aetiology, epidemiologically associated with a wholesale seafood market in Wuhan(4). On 21 January 2020, Chang et al. (5) reported the first case in Taiwan of Coronavirus Disease 19 (COVID-19) in a 55-year-old woman, after testing positive using reverse transcriptase-polymerase chain reaction (RT-PCR) to a new strain of the genus betacoronavirus(6,7), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). After the first confirmed case, the virus spread successfully worldwide and considered by the World Health Organization as pandemic (WHO) in March 2020(8).

The prompt implementation of diagnostic tests and protocols used in the virus detection represented a crucial contribution, especially RT-PCR by its proven safety in other coronavirus strains. The use of this method, together with numerous actions, proved its effectiveness in the control of COVID-19 in Chinese territory. The global SARS-CoV-2 pandemic has challenged humanity in the search for a rapid and safe solution to contain the accelerated spread of COVID-19. Accurate detection methods are needed, where RT-PCR plays a major role.

This investigation aims to describe the basis of the reverse transcriptase-polymerase chain reaction as a diagnostic test for SARS-CoV-2 detection.

METHODS

A literature review was conducted on articles published up to May 2020. A survey on the databases Scopus, Wiley Online Library, SciELO, DIALNET, EBSCO, MEDLINE and PubMed with the search terms RT-PCR test, RT-

PCR, reverse transcriptase-polymerase chain reaction, COVID-19, SARS-CoV-2, and its Spanish translations, retrieved 40 articles of interest included in this research and published in refereed journals. Boolean operators and specific structures of the search formulas for each database allowed a reduction on article retrieval.

DEVELOPMENT

A comparison between the genome of 1 008 types of SARS-CoV in humans, 338 in bats and 3 131 MERS-CoV established a high similarity with SARS-CoV-2 with only 5 nucleotide differences of approximately 29.8 kb(9). The complete genome sequence of the new coronavirus (WH-Human_1) was first published on 10 January 2020(10). A review on the genetic code of SARS-CoV-2 found differences with SARS-CoV and similar coronaviruses corresponding to 380 amino acid substitutions(9). SARS-CoV-2 has 14 ORFs encoding 27 proteins and is parallel to SARS-like bat coronaviruses(9,11,12).

The compilation of the viral code represented an essential part in the development of diagnostic tests based on the principle of RT-PCR, with the need of a set of primers to identify specific sequences associated with changes in the SARS-CoV-2 genome(13) and to avoid cross-reaction with other viral strains of the same family or other respiratory pathogens.

Basic principles and high performance tests.

Real-time quantitative RT-PCR detects and quantifies specific nucleic acid sequences using fluorescent reporters. Among the commercially available probe technologies widely used in diagnostic packages, TaqMan and Molecular beacon are the major techniques(14). The term real-time represents that the detection of the amplified products occurs in each cycle of the reaction. The term quantitative determines that it is possible to quantify the amount of DNA in the sample. The advantage over conventional PCR is that the amplification product is monitored as the reaction proceeds, without the need of an agarose gel (15).

Diagnostic packages of RT-PCR for the SARS-CoV-2 detection target the RNA dependent polymerase (RdRp), ORF1ab frames, the envelope gene (E gene), the nucleocapsid protein gene (N gene)(13,16,17,18,19,20) and the S gene(11,16). To improve detection sensitivity, most manufacturers choose two or more target regions of the viral nucleic acid sequence(11,13,21). The diagnosis is confirmed in patients with positive results for both ORF1ab gene amplification and N or E gene amplification(18). One-step RT-PCR targeting ORF1b fragments or the N gene of SARS-CoV-2 is designed to react with SARS-CoV and closely related viruses, such as MERS coronavirus, which can lead to false-positive reactions in the diagnosis of SARS-CoV-2(22).

The German company TIB MOLBIOL GmbH, in collaboration with several partners, developed a real-time RT-PCR test for the second week of January 2020. The test detects viral RNA reading the E-gene and RdRp(23). Specific for SARS-CoV-2 RNA (Gene E: 3.2 RNA copies/ 95 % CI: 2.2-6.8) (RdRP 3.7 RNA copies/ 95 % CI: 2.8-8.0) and did not cross-react with other coronaviruses(24) . In another approach, the researchers created

single-step RT-PCR assays to detect the ORF1b frames and N-gene regions of SARS-CoV-2 in one hour and 15 minutes(23).

Chan et al. (25) designed an RT-PCR assay targeting RdRp/Helicase, which did not cross-react with other coronaviruses and proved higher analytical sensitivity (11.2 copies/reaction of RNA transcripts) compared to the RdRp-P2 assay, which exhibited 42 false-negative results with an average viral load of 3.21×10^4 RNA copies/ml.

High-performance commercially available tests include the Xpert® Xpress SARS-CoV-2 test by Cepheid, USA. This test provides results in 45 minutes using the GenXpert benchtop system. The test requires one minute for sample preparation and targets multiple regions of the viral genome (N gene and E gene). In clinical samples, Xpert Xpress SARS-CoV-2 achieved a 100% match compared to other developed RT-PCRs, and the assay outperformed the commonly used diagnostic platforms in the sensitivity panel with a detection limit of 8.26 x 10^1 copies/mL(26).

Results variation according samples.

Nasopharyngeal swabs are the principal collection methods in the RT-PCR test but may miss an early-stage infection, in which case a deeper sample obtained by bronchoscopy is suitable(27). Bronchial specimens easily detect SARS-CoV-2 nucleic acid in the alveolar lavage fluid, followed by nasal and pharyngeal swabs(11). Liu et al (28) reported in a study of 4880 cases that the alveolar lavage fluid reached the 100 % positive rate for the ORF1ab frames of SARS-CoV-2 followed by sputum (49.12 %) and nasopharyngeal swab samples (38.25 %).

Wang et al. (29) reported that during the COVID-19 outbreak in China, oropharyngeal swabs reached a higher proportion compared to nasopharyngeal swabs, however, SARS-CoV-2 RNA achieved 32% detection using RT-PCR in swabs with oropharyngeal samples, which was significantly lower than the 63% positivity in the nasal swabs. To corroborate the results, another study performed compared both samples showing that the 73.1 % of positive cases using nasopharyngeal swabs, were negative on the oropharyngeal specimens(30), indicating that false negatives can occur using only samples from the oropharyngeal cavity(19,30).

Detection of SARS-CoV-2 RNA in lower respiratory samples (sputum or endotracheal aspiration) is reported in 100 % of cases, nasal mucosa in 81 %, faeces in 69 %, oropharynx in 63 %, gastric content in 46 %, anal mucosa in 25 %, conjunctiva in 6.7 %, and urine in 6.2 %(31). Furthermore, Wang et al. (29) concluded that bronchoalveolar lavage fluid presented the highest positivity rates (93%), followed by sputum (72%) and nasal swabs (63%).

Positivity of RT-PCR during time.

In most individuals with symptomatic COVID-19 infection, the viral RNA in the nasopharyngeal swab is detected from the first day of symptoms and peaks after one week(13). In a study by Wölfel et al. (32), all

patients examined between day one and day five tested positive for the virus, while none of the 27 urine samples and 31 serum samples were positive for SARS-CoV-2 RNA. Tang-Xiao et al. (19) estimated that the average time from symptom onset to a negative SARS-CoV-2 RT-PCR test result is 20 days. In some cases, viral RNA is detected by RT-PCR six weeks after the first positive test(13).

Nonconventional samples for the diagnostic.

Patients with advanced-stage COVID-19 pneumonia show a high viral RNA load for SARS-CoV-2 when stool samples are tested, as well as a lower presence of the virus in the respiratory tract. In previous outbreaks of coronaviruses that caused epidemic events, enteric involvement in transmission emerged. Therefore screening of rectal swab samples should be considered for SARS-CoV-2 in advanced cases of COVID-19(33,34).

Wang et al. (35) reported three cases discharged meeting all the criteria approved by the National Health Commission of the People's Republic of China and subsequently readmitted as positive cases of the virus. The three patients presented mainly gastrointestinal symptoms such as diarrhoea and changes in bowel habits, and also tested positive for faecal samples after an initial negative result for respiratory samples.

The authors consider as required the inclusion of diagnostic tests, using rectal swab collections in cases where the respiratory tract samples are negative, and the patient maintains the symptoms of COVID-19.

Salivary glands express the surface receptor of angiotensin II converting enzyme (ACE2); it is determined that the entry of SARS-CoV-2 into the cell depends on its binding to the ACE2 receptor(36). Azzi et al. (37) collected in Italy salivary samples from 25 patients affected by COVID-19; the tested samples resulted positive for all patients. In a similar study conducted by Williams et al. (38) in Australia, positive samples comprised 33 of 39 patients infected with the virus. However, greater sensitivity is reported using nasopharyngeal swabs.

Automatization sensibility in RT-PCR tests.

The dependence on manual settings in the RT-PCR test is one of the fundamental limitations during SARS-CoV-2 molecular diagnostics, specifically when it comes to scalability and speed in outbreak scenarios. Therefore, alternative workflows are required to allow rapid tracking of high-priority samples. A fully automated RT-PCR platform, which performs extraction, amplification and detection of viral genetic material without the need for human interaction could be the solution, such as the NeuMoDx 96 system or the Cobas 6800 SARS-CoV-2 test (39,40).

Diagnostic results from the Cobas 6800 SARS-CoV-2 automated platform using the Universal Media Transport System (UTM-RT) registered an overall agreement of 98.1% (211/215; 95% CI, 95.0-99.4%) compared to the LightMix diagnostic package(40), also the NeuMoDx 96 automated platform compared to the Cobas 6800 SARS-CoV-2 test exhibited a 100% positive match (35/35) and a 99.2% negative match (129/130)(39). The superiority

of these systems over conventional tests lies in the reduced time required for sample processing and the mitigation of errors in the workflow.

Although the automation of the whole RT-PCR process (including RNA purification), is not feasible in all countries, mainly due to its high price, the adaptation of strategies to speed up the manual analysis of samples might be a useful alternative.

Reverse transcription loop-mediated isothermal (RT-LAMP) assay.

Another method to achieve effective identification and isolation of SARS-CoV-2 is through a rapid diagnostic test, which can be performed in the field and local care facilities, without the need for specialized equipment or highly trained professionals to interpret the results. The RT-LAMP test is suitable for this purpose(41,42).

This diagnostic test provided positive results within 26.28 ± 4.48 min(46), while the RT-PCR assay requires one to two hours after viral RNA preparation to obtain a result. It uses the same principle of polymerase chain reaction (PCR), but RT-LAMP does not require the thermal cycles that increase the DNA replication like in RT-PCR and has the advantage of running at a constant temperature between 60 and 65ºC. Lin et al. (43) conducted a study with 130 swabs and samples of bronchoalveolar lavage fluid, the RT-LAMP assay resulted in 58 confirmed individuals and no cross-reactivity with other respiratory pathogens.

Yan et al. (41) developed a set of orf1ab-4 and S-123 primers with RT-LAMP technology that delivered positive results in 18 ± 1.32 min and 20 ± 1.80 min times, respectively. The sensitivity of the assays was 2×10^1 copies/reaction for orf1ab-4 and 2×10^2 copies/reaction for S-123, both developed at a temperature of 63ºC and for 60 min. Using 60 strains of human respiratory pathogens the specificity only cross-reacted with the pseudoviruses, therefore, the RT-LAMP assay did not show any cross-reactivity with other respiratory pathogens.

Gun-Soo et al.(42) validated an RT-LAMP assay within 30 minutes time results after the onset of the amplification reaction, which was optimized by the Leuco Crystal Violet colorimetric detection method.

The main limitations of RT-PCR and other tests based on PCR principles are related to mutations in the specific region of the target gene selected for the development of primers. Therefore, it is necessary to monitor the mutant sites in the virus genome through a complete sequence, in search of possible variations and include them in new primers for the validation of new assays.

CONCLUSIONS

The availability of diagnostic tests is crucial for the isolation of positive cases and the traceability of the transmission chain, where the reverse transcriptase-polymerase chain reaction roles a major part. This test exhibits the highest indicators of sensitivity and specificity. The sample that offers better results is the

nasopharyngeal swab. The need for rectal swabs samples has been documented in false-negative cases that maintain symptoms suggestive of COVID-19. The automation and optimization of diagnostic tests are continuously changing, where the reverse transcription loop-mediated isothermal amplification test (RT-LAMP) is an effective alternative.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

José Francisco Cancino Mesa: Conceptualization, Data curation, Resources (Equal), Visualization (Equal), Writing original draft, Writing – review & editing (Equal).
Adrián Alejandro Vitón Castillo: Methodology, Resources (Equal), Visualization (Equal), Writing – review & editing (Equal).
Jorge Casí Torres: Resources (Equal), Supervision, Visualization (Equal), Writing – review & editing (Equal).

FUNDING

The authors did not receive funding for the development of this research.

REFERENCES

1. Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. *Journal of Medical Virology* [Internet]. 2020 [cited 12 mayo 2020]; 92(4): 418-423. Available from: http://dx.doi.org/10.1002/jmv.25681

2. de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ. Host factors in coronavirus replication. En* Springer, Cham. Roles of Host Gene and Non-coding RNA Expression in Virus Infection. Vol.419. Springer, Cham; 2017 p.1-42. Available from: https://doi.org/10.1007/82_2017_25

3. Paules CI, Marston HD, Fauci AS. Coronavirus infections - more than just the common cold. *JAMA* [Internet]. 2020 [cited 12 mayo 2020]; 323(8): 707-708. Available from: http://dx.doi.org/10.1001/jama.2020.0757

4. Arshad-Ali S, Baloch M, Ahmed N, Arshad-Ali A, Iqbal A. The outbreak of Coronavirus Disease 2019 (COVID-19) - An emerging global health threat. *Journal of Infection and Public Health* [Internet]. 2020 [cited 15 mayo 2020]; 13(2020): 644-646. Available from: https://www.sciencedirect.com/science/article/pii/S1876034120303658

5. Shao-Chung C, Yuan-Chia C, Yu-Long FC, Yu-Chan C, Mingte C, Yang CH et al. First case of Coronavirus Disease 2019 (COVID-19) pneumonia in Taiwan. *Journal of the Formosan Medical Association* [Internet]. 2020 [cited 27 abril 2020]; 119(3): 747-751. Available from: https://www.sciencedirect.com/science/article/pii/S0929664620300449

6. J Reina. El SARS-CoV-2, una nueva zoonosis pandémica que amenaza al mundo. *Vacunas* [Internet] 2020 [cited 21 mayo 2020];21(1): 17-22. Available from: https://doi.org/10.1016/j.vacun.2020.03.001

7. Moreno Martínez FL, Moreno López FL, Oroz Moreno R. Repercusión cardiovascular de la infección por el nuevo coronavirus SARS-CoV-2 (COVID-19). *CorSalud*. 2020 [cited 21 mayo 2020]; 12(1):3-17. Available from: http://www.revcorsalud.sld.cu/index.php/cors/article/view/588/1112

8. Adhanom-Ghebreyesus T. WHO Director-General's opening remarks at the media briefing on COVID-19 [Internet]. WHO; 2020 [cited 27 abril 2020]. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

9. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. *Cell Host & Microbe* [Internet]. 2020. [cited 2 mayo 2020]; 27(3): 325-328. Available from: https://www.sciencedirect.com/science/article/pii/S193131282030072X

10. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG et al. A new coronavirus associated with human respiratory disease in China. *Nature* [Internet]. 2020 [cited 12 mayo 2020]; 579(2020): 265–269 Available from: https://www.nature.com/articles/s41586-020-2008-3

11. Wang H, Li X, Li T, Zhang S, Wang L, Wu X et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *J Clin Microbiol Infect Dis* [Internet]. 2020 [cited 5 mayo 2020]. Available from: https://dx.doi.org/10.1007/s10096-020-03899-4

12. Khailany R, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. *Gene Reports* [Internet]. 2020 [cited 5 mayo 2020]; 19(Junio 2020): 100682. Available from: https://www.sciencedirect.com/science/article/pii/S2452014420300960

13. Sethuraman N, Stanleyraj-Jeremiah S, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2 *JAMA* [Internet]. 2020 [cited 18 mayo 2020]; 323(22):2249–2251. Available from: https://doi.org/10.1001/jama.2020.8259

14. Wang-Shick Ryu. Molecular virology of human pathogenic viruses. London, UK ; San Diego, CA : Academic Press; 2017.

15. Tamay de Dios L, Ibarra C, Velasquillo C. Fundamentos de la reacción en cadena de la polimerasa (PCR) y de la PCR en tiempo real. *Investigación en discapacidad* [Internet]. 2013 [cited 12 Jul 2020]; 2(2): 70-78. Available from: https://www.medigraphic.com/pdfs/invdis/ir-2013/ir132d.pdf

16. Lorussoa A, Calistria P, Mercantea MT, Monacoa F, Portanti O, Marcacci M et al. A "One-Health" approach for diagnosis and molecular characterization of SARS-CoV-2 in Italy. *One Health* [Internet]. 2020 [cited 5 mayo 2020]; 10 (2020): 100135. Available from: https://www.sciencedirect.com/science/article/pii/S2352771420300823

17. Ding-feng L, Qi-ming Ying, Yue-song Weng, Chi-bin Shen, Jin-guo Chu, Kong J et al. Dynamic change process of target genes by RT-PCR testing of SARS-Cov-2 during the course of a Coronavirus Disease 2019 patient. *Clinica Chimica Acta* [Internet]. 2020 [cited 5 mayo 2020]; 506(Julio 2020): 172-175. Available from: https://www.sciencedirect.com/science/article/pii/S0009898120301340

18. Wang M, Zhou Y, Zong Z, Liang Z, Cao Y, Tang H et al. A precision medicine approach to managing Wuhan Coronavirus pneumonia. *Precision Clinical medicine* [Internet] . 2020 [cited 8 mayo 2020]; 3(1) 14-21. Available from: https://doi.org/10.1093/pcmedi/pbaa002

19. Tang-Xiao A, Xin-Tong Y, Gao C, Zhu L, Jie-Zhang Y, Zhang S. Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: A descriptive study. *Journal of Clinical Virology* [Internet]. 2020 [cited 12 mayo 2020]; 127(Junio 2020): 104346. Available from: https://doi.org/10.1016/j.jcv.2020.104346

20. Hogan C, Sahoo M, Hong-Huang C, Garamani N, Stevens B. Comparison of the Panther Fusion and a laboratory-developed test targeting the envelope gene for detection of SARS-CoV-2. *Journal of Clinical Virology* [Internet]. 2020 [cited 12 mayo 2020]; 127 (Junio 2020): 104383. Available from: https://doi.org/10.1016/j.jcv.2020.104383

21. Ishigea T, Murataa S, Taniguchib T, Miyabea A, Kitamuraa K, Kawasaki K et al. Highly sensitive detection of SARS-CoV-2 RNA by multiplex rRT-PCR for molecular diagnosis of COVID-19 by clinical laboratories. *Clinica Chimica Acta* [Internet]. 2020 [cited 5 mayo 2020]; 507(agosto 2020): 139-142. Available from: https://www.sciencedirect.com/science/article/pii/S0009898120301789

22. Li C, Ren L. Recent progress on the diagnosis of 2019 Novel Coronavirus. *Transboundary and Emerging Diseases* [Internet]. 2020 [cited 18 mayo 2020]; 67(4): 1485-1491. Available from: https://doi.org/10.1111/tbed.13620

23. Kumar-Vashist S. In Vitro Diagnostic Assays for COVID-19: Recent Advances and Emerging Trends. *Diagnostics* [Internet]. 2020 [cited 5 mayo 2020]; 10(4):202. Available from: https://www.mdpi.com/2075-4418/10/4/202

24. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu D, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* [Internet]. 2020 [cited 21 mayo 2020]; 25(3):2000045. Available from: https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

25. Fuk-Woo-Chan J, Chik-Yan-Yip C, Kai-Wang-To K, Hing-Cheung-Tang T, Cheuk-Ying-Wong S, Leung KH et al. Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens. *Journal of Clinical Microbiology* [Internet]. 2020 [cited 18 mayo 2020]; 58:e00310-20. Available from: https://jcm.asm.org/content/58/5/e00310-20.long

26. Wolters F, Van de Bovenkamp J, Van den Bosch B, Van den Brink S, Broeders M, Chung-Hoa N et al. Multi-center evaluation of cepheid xpert® xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic. *Journal of Clinical Virology* [Internet] 2020 [cited 14 mayo 2020];128(Julio 2020): 104426. Available from: https://dx.doi.org/10.1016/j.jcv.2020.104426

27. Loeffelholz M, Wei-Tang Y. Laboratory diagnosis of emerging human coronavirus infections– the state of the art. *Emerging Microbes & Infections* [Internet]. 2020. [cited 5 mayo 2020]; 9(1): 747-756. Available from: https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1745095

28. Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta* [Internet]. 2020 [cited 8 mayo 2020]; 505(Junio 2020): 172–175. Available from: https://doi.org/10.1016/j.cca.2020.03.009

29. Wang W, Xu Y, Gao R, Gao R, Lu R, Han K, Wu G et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* [Internet] . 2020 [cited 7 mayo 2020]; 323(18) 1843-1844. Available from: https://doi.org/10.1001/jama.2020.3786

30. Wang X, Tan L, Wang X, Liu W, Lu Y, Cheng L et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. *International Journal of Infectious Diseases* [Internet]. 2020 [cited 5 mayo 2020]; 94(Mayo2020): 107-109. Available from: https://www.sciencedirect.com/science/article/pii/S1201971220302356

31. Huang Y, Chen S, Yang Z, Guan W, Liu D, Lin Z, et al. SARS-CoV-2 Viral Load in Clinical Samples of Critically Ill Patients. *Am J Respir Crit Care Med* [Internet]. 2020 [cited 5 mayo 2020]; 201(11). Available from: https://doi.org/10.1164/rccm.202003-0572le

32. Wölfel R, Corman V, Guggemos W, Seilmaier M, Zange S, Müller MA et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* [Internet]. 2020 [cited 18 mayo 2020]; 581(2020): 465–469. Available from: https://dx.doi.org/10.1038/s41586-020-2196-x

33. Wei-Tang Y, Schmitz J, Persing D, Stratton C. The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges. *Journal of Clinical Microbiology* [Internet]. 2020 [cited 5 mayo 2020]; 58(6) e00512-20. Available from: https://jcm.asm.org/content/early/2020/04/03/JCM.00512-20

34. Zhang T, Cui X, Zhao X, Wang J, Zheng J, Zheng G et al (2020) Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. *Journal of Medical Virology* [Internet]. 2020 [cited 10 mayo 2020]; 92(7): 909-914. Available from: https://doi.org/10.1002/jmv.25795

35. Wang X, Zhou Y, Jiang N, Zhou Q, Ma W-L. Persistence of intestinal SARS-CoV-2 infection in patients with COVID-19 leads to re-admission after pneumonia resolved. *International Journal of Infectious Diseases* (2020) [Internet]. 2020 [cited 12 mayo 2020]; 95(2020): 433-435. Available from: https://doi.org/10.1016/j.ijid.2020.04.063

36. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* [Internet]. 2020 [cited 5 mayo 2020]; 181:(2) 271-280.e8. Available from: https://www.sciencedirect.com/science/article/pii/S0092867420302294

37. Azzi L, Carcano G, Gianfagna F, Grossie P, Dalla-Gasperina D, Genoni A et al. Saliva is a reliable tool to detect SARS-CoV-2. *Journal of Infection* [Internet]. 2020 [cited 5 mayo 2020];81(1): e45-e50 . Available from: https://www.sciencedirect.com/science/article/pii/S0163445320302139

38. Williams E, Bond K, Zhang B, Putland M, Williamson D. Saliva as a non-invasive specimen for detection of SARS-CoV-2. *Journal of Clinical Microbiology* [Internet]. 2020 [cited 5 mayo 2020]; [In Press]:JCM.00776-20 . Available from: https://jcm.asm.org/content/jcm/early/2020/04/17/JCM.00776-20.full.pdf

39. Nörz D, Fischer N, Schultze A, Kluge S, Mayer-Runge U. Clinical evaluation of a SARS-CoV-2 RT-PCR assay on a fully automated system for rapid on-demand testing in the hospital setting. *Journal of Clinical Virology* [Internet]. 2020 [cited 12 mayo 2020]; 128 (Junio 2020): 104390. Available from: https://doi.org/10.1016/j.jcv.2020.104390

40. Poljak M, Korva M, Knap-Gašper N, Fujs-Komloš K, Sagadin M. Clinical evaluation of the cobas SARS-CoV-2 test and a diagnostic platform 2 switch during 48 hours in the midst of the COVID-19 pandemic. *Journal of Clinical Microbiology* [Internet]. 2020 [cited 12 mayo 2020] 58:e00599-20. Available from: http://doi.org/10.1128/JCM.00599-20

41. Yan C, Cui J, Huang L, Du B, Chen L, Xue G et al. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clinical Microbiology and Infection* [Internet]. 2020 [cited 5 mayo 2020]; 26(6): 773-779. Available from: https://www.sciencedirect.com/science/article/pii/S1198743X20301865

42. Gun-Soo, Keunbon P, Seung-Hwa K, Seong-Jun B, Seung K, Kim BT et al. Development of Reverse Transcription Loop-Mediated Isothermal Amplification Assays Targeting Severe Acute Respiratory Syndrome Coronavirus 2. *The Journal of Molecular Diagnostics* [nternet]. 2020 [cited 5 mayo 2020]; 22(6): 729-735. Available from: https://www.sciencedirect.com/science/article/pii/S1525157820300908

43. Lin Y, Wu S, Hao X, Li X, Liu X, Ye S et al. Rapid Detection of COVID-19 Coronavirus Using a Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Platform. *Clinical Chemistry* [Internet]. 2020 [cited 13 julio 2020]; 66(7): 975-977. Available from: https://academic.oup.com/clinchem/article/66/7/975/5823294