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REVIEW ARTICLE

The polymerase chain reaction-based SARS-CoV-2 detection

Empleo de la reacción en cadena de la polimerasa en la detección del SARS-CoV-2

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ABSTRACT

Introduction: the reverse transcriptase polymerase chain reaction is a highly precise technique in the detection and amplification of genetic material, and therefore in the detection of viruses.
Objective: to describe the bases of the use of the polymerase chain reaction with reverse transcriptase as a diagnostic test in the detection of SARS-CoV-2.
Method: a literature review was carried out on articles published up to May 2020. The following databases were consulted: Scopus, Wiley Online Library, SciELO, DIALNET, EBSCO, MEDLINE and PubMed. Articles in Spanish and English were retrieved, selecting 43 references.
Development: The reverse transcriptase polymerase chain reaction to detect SARS-CoV-2 consists of reading RNA-dependent RNA polymerase, ORF1ab fragments, the E gene, the N gene, and the S gene. The nasopharyngeal exudate offers better results than oropharyngeal and saliva as sample. The inclusion of reverse transcriptase polymerase chain reaction tests using rectal swab specimens is necessary in suspected false negative cases. New studies and techniques are developed with the aim of optimizing the detection process.
Conclusions: the availability of diagnostic tests is crucial for the isolation of positive cases and the monitoring of the epidemiological chain of transmission. RT-PCR turned out to be the test of choice during the viral replication period. The RT-LAMP assay is a rapid diagnostic alternative with similar principles to RT-PCR.

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

RESUMEN

Introducción: la reacción en cadena de la polimerasa con transcriptasa inversa es una técnica de alta precisión en la detección y amplificación de material genético, y por ende en la detección de virus.
Objetivo: describir las bases del empleo de la reacción en cadena de la polimerasa con transcriptasa inversa como prueba diagnóstica en la detección del SARS-CoV-2.
Método: se realizó una revisión de la literatura en artículos publicados hasta mayo de 2020. Se consultaron las bases de datos: Scopus, Wiley Online Library, SciELO, DIALNET, EBSCO, MEDLINE y PubMed. Se recuperaron artículos en español e inglés, seleccionándose 43 referencias.
Desarrollo: la reacción en cadena de la polimerasa con transcriptasa inversa para detectar SARS-CoV-2 consiste en la lectura de la ARN polimerasa dependiente del ARN, fragmentos ORF1ab, el gen E, el gen N y el gen S. El exudado nasofaríngeo ofrece mejores resultados que el orofaríngeo y saliva como muestra. Resulta necesaria la inclusión de pruebas reacción en cadena de la polimerasa con transcriptasa inversa

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que utilicen especímenes de hisopado rectal en casos sospechosos falsos negativos. Nuevos estudios y técnicas se elaboran con el objetivo de optimizar el proceso de detección.
Conclusiones: la disponibilidad de pruebas diagnósticas es crucial para el aislamiento de casos positivos y el seguimiento de la cadena epidemiológica de transmisión. La RT-PCR resultó ser la prueba de elección durante el período de replicación viral. El ensayo RT-LAMP es una alternativa diagnóstica rápida con principios similares a la RT-PCR.

Palabras clave: Infecciones por Coronavirus; Reacción en Cadena de la Polimerasa; Técnicas de Laboratorio Clínico.

INTRODUCTION

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

In 2002, there was an outbreak of severe acute respiratory syndrome (SARS) caused by SARS-CoV, which endangered the lives of 8 098 people with a mortality of 774 patients. The epicenter of the disease 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, Middle Eastern Respiratory Syndrome (MERS), caused by MERS-CoV, emerged in Saudi Arabia. Bats were considered the natural hosts and the intermediate hosts were dromedary camels. A total of 2494 cases, with 858 deaths, were reported to be due to rapid nosocomial transmission. MERS demonstrated clinical features similar to SARS with prominent gastrointestinal symptoms and acute renal failure.[3,4]

In December 2019, the Chinese government warned the international scientific community of a sporadic outbreak of pneumonia cases with no known etiology, epidemiologically associated with a wholesale seafood market in Wuhan.[4] On January 21, 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 for the oropharyngeal swab collection using the reverse transcriptase polymerase chain reaction (RT-PCR) technique applied to detect the new strain of the genus betacoronavirus,[6,7] Coronavirus-2 of the Severe Acute Respiratory Syndrome (SARS-CoV-2). After the first confirmed positive case was reported, the virus spread rapidly worldwide and was considered a pandemic by the World Health Organization (WHO) on March 2020.[8]

The rapid development of diagnostic tests and protocols used to detect the virus was a crucial contribution, especially RT-PCR because of its proven safety in other strains of coronavirus. The use of this method, together with numerous actions, demonstrated the effectiveness in the control of COVID-19 in Chinese territory. The global SARS-CoV-2 pandemic has tested humanity in the search for a fast and safe solution to contain the rapid spread of COVID-19. Until the definitive cure is identified, it is necessary to have precise detection means, where for the moment; RT-PCR seems to be the best bet.

The aim of this research is to describe the basis for the use of reverse transcriptase polymerase chain reaction as a diagnostic test in the detection of SARS-CoV-2 (COVID-19).

METHOD

A literature review was conducted on articles published up to May 2020. The databases Scopus, Wiley Online Library, SciELO, DIALNET, EBSCO, MEDLINE and PubMed were consulted.

Data collection was carried out during the months of April and May. The terms used in the search were: RT-PCR test, RT-PCR, reverse transcriptase polymerase chain reaction, COVID-19, SARS-CoV-2, and its English

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translations "RT-PCR test", "RT-PCR", "reverse transcriptase polymerase chain reaction", "COVID-19", "SARS-CoV-2".

The terms were used in search formulas, using Boolean operators. The structures of the search formulas were database-specific. Forty articles published in refereed journals belonging to the field of Health Sciences were selected.

DEVELOPMENT

Comparisons made between the genome of 1 008 types of SARS coronavirus in humans, 338 in bats and 3 131 MERS coronavirus, allowed establishing a great similarity with SARS-CoV-2 with only 5 differences in nucleotides of approximately 29,8kb.[9] The complete genome sequence of the new coronavirus (WH-Human_1) was first published on January 10, 2020.[10] A review of the SARS-CoV-2 genetic code showed that the differences with SARS-CoV and similar coronaviruses correspond 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 complete obtaining of the viral code constituted a fundamental contribution in the development of diagnostic tests based on RT-PCR principle, given the need of primers synthesis that allowed a correct identification of specific sequences of base pairs associated to changes in SARS-CoV-2 genome,[13] with the aim of avoiding crossed reaction with other viral strains of the same family or respiratory pathogens.

Basic operation and high performance testing

Real-time quantitative RT-PCR detects and quantifies specific nucleic acid sequences using fluorescent reporters. Among the commercially available probe technologies, those most widely used in diagnostic packages include TaqMan and Molecular beacon technologies.[14] The term quantitative refers to the fact 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 for it to be manipulated in an agarose gel to know if the reaction was successful, as is the case in endpoint PCR.[15]

Diagnostic packages employing RT-PCR for the detection of SARS-CoV-2 work by reading RNA-dependent polymerase (RdRp), ORF1ab fragments, the envelope gene (E gene), the nucleocapsid protein gene (N gene),[13,16,17,18,19,20] and the S gene.[11,16] In order 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 the ORF1ab gene and the N or E gene amplification.[18] One-step RT-PCR targeting ORF1b fragments or the N gene of SARS-CoV-2 was designed to react with SARS-CoV and closely related viruses, such as MERS coronavirus, which can lead to false positive reactions in the identification of the virus causing the COVID-19.[22]

The German company TIB MOLBIOL GmbH, in collaboration with several partners developed a novel and robust real-time RT-PCR test for the second week of January 2020. The test detects viral RNA by means of E- envelope and RdRp genetic assays.[23] It proved to be very 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) and did not cross react with other coronaviruses.[24] In another study approach, the researchers created single-step RT-PCR assays to detect the ORF1b and N-gene regions of SARS-CoV-2 in 1 h and 15 min.[23]

Chan et al.[25] prepared a RT-PCR assay targeting SARS-CoV-2 RNA-dependent polymerase/heliase (RdRp/Hel), which did not cross react with other coronaviruses and demonstrated increased analytical sensitivity(11,2 copies/reaction with in vitro RNA transcripts) compared to the RdRp-P2 assay, which yielded 42 false negative results with an average viral load of 3,21 × 104 RNA copies/ml.

Among the high-performance commercially available tests is the Xpert® Xpress SARS-CoV-2 test by Cepheid, USA. This test provides results in only 45 minutes using the GenXpert bench top system. The test requires one minute for sample preparation and targets multiple regions of the viral genome. In clinical samples, Xpert Xpress SARS-CoV-2 achieved a 100 % match compared to other RT-PCRs developed, and the assay outperformed

the diagnostic platforms commonly used in the sensitivity panel with a detection limit of 8,26 x 101 copies / mL.[26]

Variation of results according to samples

Nasopharyngeal swab is usually the collection method used when making the diagnosis by RT-PCR, but it can miss an infection in initial stages, in these cases a deeper sample obtained by bronchoscopy is useful.[27] The bronchial sample has the advantage of detecting more easily the viral nucleic acid in the alveolar lavage fluid, followed by nasal and pharyngeal sputum swabs.[11]

In a study of 4 880 cases, Liu et al. [28] showed that the alveolar lavage fluid exhibited the 100 % positive rate for the ORF1ab fragment of SARS-CoV-2; sputum exhibited a positive rate of 49,12 %, for nasal and pharyngeal sputum swabs showed a low positive rate of 38,25 %.

Wang et al.[29] reported that oropharyngeal swabs were used much more frequently than nasopharyngeal swabs during the SARS-CoV-19 outbreak in China; however, SARS-CoV-2 RNA was detected in only 32 % of the swabs with oropharyngeal samples, which was significantly lower than the 63 % positivity in the nasal swabs. To corroborate the results, another study was conducted comparing both samples were 73,1 % of the positive cases using nasopharyngeal swabs were negative on the oropharyngeal swab[30], indicating that false negatives can occur using only the oropharyngeal swab.[19,30]

In a number of patients, detection of virus RNA in lower respiratory tract samples (sputum or endotracheal aspiration) has been reported in 100 % of cases, nasal mucosa (81 %), stool (69 %), oropharynx (63 %), gastric content (46 %), anal mucosa (25 %), conjunctiva (6,7 %), and urine (6,2 %).(31) On the other hand, Wang et al.(29) determined that bronchoalveolar lavage fluid samples showed the highest positive rates (93 %), followed by sputum (72 %) and nasal swab collections (63 %).

Positivity of results over 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], the swabs of all patients taken between the first and fifth day were positive for the virus, while none of the 27 urine samples and 31 serum samples were positive for SARS-CoV-2 RNA. In another study by Tang-Xiao et al.[19] the average period from the onset of symptoms to a negative SARS-CoV-2 RT-PCR test result was 20 days, and in some cases the viral RNA has been detected by RT-PCR six weeks after the first positive test.[13]

Unconventional samples for diagnosis

Patients with advanced stage COVID-19 pneumonia have demonstrated a high viral RNA load for SARS-CoV-2 when stool samples are analyzed, as well as a lower presence of the virus in the respiratory tract. In previous outbreaks of coronavirus that caused epidemic events, an enteric involvement in transmission was proven, therefore, the analysis of rectal swab collections should be considered to detect SARS-CoV-2 in advanced cases of COVID-19.[33,34]

Wang et al.[35] reported three cases that were discharged meeting all criteria approved by the National Health Commission of the People's Republic of China, and were subsequently readmitted as positive cases of the virus. The three patients presented mainly gastrointestinal symptoms such as diarrhea and changes in bowel habits; they tested positive for stool samples after testing negative for respiratory samples.

The authors consider necessary the inclusion of diagnostic tests using rectal swab collections in the cases where the samples of the respiratory system were negative and the patient maintained the symptoms suggestive of COVID-19.

The salivary glands express the surface receptor of the angiotensin II converting enzyme (ACE2); it has been determined that the entry into the cell of SARS-CoV-2 depends largely on its binding to this receptor[36]. Azzi

et al.[37] in a study carried out in Italy collected salivary samples from 25 patients affected by COVID-19; the samples were analyzed by RT-PCR and were positive for all patients. In another similar study conducted by Williams et al.[38] in Australia, positive samples were found in 33 of 39 patients infected with the virus. Although studies for the detection of SARS-CoV 2 using saliva as a sample are still insufficient, increased sensitivity has been demonstrated when using nasopharyngeal swabs.

Efficiency and automation of RT-PCR tests

The reliance on manual settings in the RT-PCR test is one of the fundamental limitations during SARS-CoV-2 molecular diagnostics when it comes to scalability and speed in outbreak scenarios. Therefore, alternative workflows are required to enable 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 approaches of the Cobas 6800 SARS-CoV-2 automatic platform using the Universal Media Transport System (UTM-RT) showed an overall agreement of 98,1 % (211/215; 95 % CI, 95-99,4 %) compared to the LightMix diagnostic package[40], while the NeuMoDx 96 automatic platform compared to the Cobas 6800 SARS-CoV-2 test showed a 100 % positive match (35/35) and a 99,2 % negative match (129/130).[39] The superiority of these systems over conventional testing lies in the reduced time required for sample processing and the elimination of possible human error.

Although the automation of the entire process is not feasible in all countries, mainly due to its high price, the adaptation of strategies that allow accelerating the manual analysis of the samples would be a useful alternative in these cases.

Reverse Transcription Loop-mediated Isothermal Amplification Test (RT-LAMP)

Another method to achieve an effective identification and isolation of SARS-CoV-2, would be given by a rapid and robust diagnostic test, which can be performed in the field and in local care centers, without the need for specialized equipment or highly trained professionals to interpret the results. This is the case of the RT-LAMP test.[41,42]

This novel test presented positive diagnostic results in a time of 26,28 ± 4,48 min[46], while RT-PCR assay requires 1-2 h after the preparation of viral RNA to obtain a result. It uses the same principle of polymerase chain reaction (PCR) but RT-LAMP does not require the thermal cycles that facilitate DNA replication used in RT-PCR, besides having the advantage of being performed at a constant temperature ranging from 60 to 65ºC. In a study conducted by Lin et al.[43] with 130 swabs and bronchoalveolar lavage fluid samples, the assay showed 58 confirmed individuals and no cross-reactivity with other respiratory pathogens.

Yan et al.[41] created a set of orf1ab-4 and S-123 primers with RT-LAMP technology that achieved positive results in 18 ± 1,32 min and 20 ± 1,80 min times, respectively. The sensitivity of the tests was 2 x 101 copies/reaction for orf1ab-4 and 2 x 102 copies/reaction for S-123, both developed at a temperature of 63ºC and for 60 min. Specificity was evaluated using 60 strains of human respiratory pathogens and only the pseudo-viruses were positive, 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 capable of detecting the presence of the virus within 30 minutes after the start of the amplification reaction, a reaction optimized by the colorimetric detection method Leucocrystal Violet (LCV).

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

CONCLUSIONES

The accessibility of diagnostic tests is crucial for the isolation of positive cases and the monitoring of the epidemiological chain of transmission, where the reverse transcriptase polymerase chain reaction is very useful. This test exhibits high indicators of sensitivity and specificity. The most sensitive sample is the nasopharyngeal swab collection, although the need to use rectal swabs in false negative cases who maintain symptoms suggestive of COVID-19 has been documented. We are working on the automation and optimization of diagnostic tests, where the loop-mediated isothermal amplification test (RT-LAMP) is an effective alternative.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. Although AAVC and JFCM are part of the editorial team of the Journal, they did not intervene in the editorial process.

AUTHOR'S CONTRIBUTION

JFCM was in charge of the conceptualization, methodology and administration of the project. JFCM, AAVC and JCT participated in the original draft, review and editing. All authors approved the final version of the manuscript.

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