

Técnicas de Diagnóstico en la Pandemia

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Diagnostic Techniques in the Pandemic

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Abstract: Coronaviruses (CoV) are a group of enveloped, single-stranded (positive-sense) RNA viruses belonging to the nidoviral order, family Coronaviridae. They are an important family of pathogens that affect the human respiratory system can produce clinical pictures ranging from the common cold, with seasonal pattern in winter, to more serious ones such as those produced by severe acute respiratory syndrome (SARS) and Near East respiratory syndrome (MERS-CoV) viruses and the latter responsible for the great global pandemic, SAR-CoV2, which causes the COVID-19 disease that has spread worldwide with very efficient transmission and a higher case fatality rate than seasonal influenza. Diagnostic tools for the detection and monitoring of COVID-19 have become an indispensable requirement to quantify the number of cases around the world and from this point to be able to take the corresponding sanitary and political measures to give a forceful response to the outbreaks that are appearing. Testing is vital clinical diagnostic tool, and the techniques available so far have been based on detection of viral genes (such as RT-PCR reference technique), detection of antibodies and detection of viral antigens. Diagnosis has enormous potential in the fight against diseases and in obtaining better health outcomes in the population. The Covid-19 pandemic focused on the preparedness needed to assess, analyze and identify the different variables of health care. With the extensive use of quantitative reverse transcription-PCR (RT-PCR) technology, it is now possible to provide accurate and timely laboratory diagnostic test results to public health experts and other stakeholders in a robust manner. This contrasts sharply with the last influenza pandemic in 2009, in which diagnosis in many clinical settings was based primarily on clinical observations, resulting in an underestimation of the actual number of deaths caused by pandemic influenza A (H1N1) 2009 virus. Several studies worldwide show that diagnostic tests are an indispensable tool in clinical practice. First, because they provide critical information at every step of the patient pathway: from prognosis, preventive studies (screening), diagnosis to monitoring disease progression and predicting responses to treatment. And also because they play an increasingly important role in providing personalized, cost-effective, value-based healthcare. Even so, the value of diagnostic information is often not fully recognized by health systems. Diagnostic research and development helps develop products and solutions that lead to more patient-centered, outcome-based care. This helps enable healthcare professionals to make the right diagnoses at the right time.

Keywords: COVID-19; RT-PCR; Isothermal amplification; Detection of SARS-CoV-2; SARS-CoV-2 diagnosis; Rapid Antigen Test, Antibody Test.

Introduction:

In recent years we have faced the worst pandemic in the last 100 years, an infectious disease emerging by a new pathogen belonging to the family of Coronaviruses, SARS-CoV-2 that causes the COVID-19 disease that has expanded worldwide with a very efficient transmission and a case fatality rate higher than that of seasonal influenza. Coronaviruses are enveloped RNA viruses that cause respiratory illnesses of varying severity, from the common cold to deadly pneumonia. Its name comes from the peaks protruding from its membranes, which resemble the corona of the sun. They can infect both animals and humans and cause diseases of the respiratory tract. Each year, at least four types of coronaviruses, such as the common cold, cause very mild infections. Most people become infected with one or more of these viruses at some point in their lives. It is very important to note that these coronaviruses that cause severe respiratory infections are zoonotic pathogens, which start in infected animals and are transmitted from animals to people. Diagnosis is a very important aspect of addressing the consequences of any deadly contagious disease. Diagnostic tests demonstrate the presence or absence of an infectious agent. Early and effective diagnosis has helped limit deaths from highly infectious and contagious diseases in the past and has been instrumental in this current pandemic. Diagnosis has an empirical role in diseases caused by any new pathogen for which the population is not pre-immune. COVID-19 is one of those infectious diseases, highly contagious and deadly, a sensitive, specific and rapid diagnosis is crucial to identify positive cases, trace their contacts, find the source of the virus and finally streamline the infection control measure. During the initial period of the epidemic, the complete sequencing of SARS-CoV-2 facilitated the design of specific primers and laboratory diagnosis of Covid-19.

Materials and Methods:

This work represents an analysis of the scientific literature referring to the diagnosis of Covid-19. This review has sought to compile the different diagnostic approaches used by academic laboratories and clinicians to diagnose COVID-19 disease from the identification of SARS-CoV-2 until now.

Diagnosis of Covid-19:

The microbiological diagnosis of SARS-CoV-2, an agent of COVID-19 (2019 novel coronavirus disease) is important for both the management of the individual disease and the current pandemic. Although the procedure of choice (Gold Standard) is PCR, it is also necessary to have fast, simple tests with high sensitivity and precision. These techniques can be replicated on a large scale and are inexpensive. The ultimate goal is an early diagnosis, for better management (isolation and treatment if necessary of the disease), monitoring of patients, and the application of prevention and control measures of the expansion of outbreaks with better epidemiological surveillance.

Before entering the field of testing, it is necessary to talk about the pre-analytical stage, which will determine the final effectiveness of the test. At this stage we include the sampling, transport and conservation, which are very important points to maintain adequate performance and finally obtain a reliable result. It should be considered that each test has differences in its performance according to the days of evolution of the clinical picture and that it is especially critical that the quality of the sample is the best possible, following the recommendations for obtaining it and using the appropriate inputs to avoid that this generates false negative results.

Types of tests:

The tests available to detect the presence of COVID-19 are: Reverse transcriptase: polymerase chain reaction (PCR):

The reverse transcriptase polymerase chain reaction test (RT-PCR or qRT-PCR if quantified in real time) is a molecular technique for detecting and amplifying nucleic acids, i.e. genetic material, RNA, of SARS-CoV-2 in different clinical biological samples. It is currently the reference and choice technique for the diagnosis of COVID-19, as it is the most sensitive of the available methods. WHO recommends nasopharyngeal and pharyngeal gold samples in the same tube to increase viral load and thus enhance the effectiveness of the test. The complexity of this test is that it is necessary trained and prepared personnel for its use, it is very specific, so not all health workers are prepared, and it is also early because viruses are detected in the early stages of respiratory infection. It is a process that takes several hours to perform and finally know the result, which can be a disadvantage on certain occasions. The most commonly used target genes for the detection of SARS-CoV-2 are the E gene (recommended by the WHO as first-line screening), the RdRp gene, for confirmation study and the N gene for additional confirmatory study. Another gene used is Orf1ab. For the confirmation diagnosis in areas without circulation of the COVID-19 virus, positivity is needed against two different COVID-19 genes, one of them specific to it, or positivity against a beta coronavirus plus at least partial identification of the genome of the COVID-19 virus. In areas of community transmission in the country at present, rRT-PCR positivity is considered sufficient for a single gene that is discriminatory against COVID-19.

RT-PCR is a very sensitive detection tool in molecular diagnostics. It can detect and amplify even a few copies of a specific genomic sequence in a variety of samples, but relies on certain aspects to provide reliable results, such as proper collection, transport, storage, and processing of samples. RT-PCR-based kits are very expensive and take sometime to deliver results, so it is sometimes essential to look for other methods of rapid and reliable diagnostics.

Other nucleic acid-based diagnostic techniques Nucleic acid sequence-based: amplification (NASBA):

This is an *in vitro* amplification process performed under isothermal conditions. It is an amplification process consisting of two steps, where the first step is denaturation and the second step is an isothermally performed polymerase-dependent amplification. Fluorochromes are also added to the reaction to make it a real-time based observation. This technique has been further modified as a multiplex process called real-time nucleic acid sequence amplification multiplex (RT-NASBA), which can aid in the simultaneous detection of different viral infections. RT-NASBA has been shown to be 10 to 100 times more sensitive than RT-PCR Multiplex, due to isothermal conditions where no time is consumed to heat and cool and copy production is faster than RT-PCR. RT-NASBA has previously been used for the detection of SARS-CoV infections and its sensitivity and specificity were observed to parallel the diagnosis of RT-PCR. This technique may be an option for rapid diagnosis of COVID-19 during the current pandemic.

Loop-mediated isothermal amplification (LAMP):

LAMP is a comparatively less expensive, much more sensitive and faster diagnostic technique than RT-PCR. This technique involves the selective amplification of target nucleic acids at a constant temperature, usually 60 °C. In this technique, 4 to 6 primers specifically designed to detect different nucleic acid sequences are used; In addition, no initial denaturation of the template is required and reaction time is minimized up to 30 minutes using chain displacement polymerases. For colorimetry-based analysis, the LAMP reaction mixture is added with hydroxynaphthol blue (HNB) prior to amplification, thus preventing cross-contamination in the future. The time required for this assay was about an hour, considerably less than RT-PCR. The LAMP technique avoids the use of expensive reagents and instruments, helping to reduce the cost of diagnosis with quick results. Several studies have highlighted the application of the LAMP technique in the detection of coronavirus infections in patient samples. It was also observed that 9 to 10 copies of viral RNA per reaction were sufficient to detect the infection giving a sensitivity 100 times greater than RT-PCR. In addition, this method has been integrated into a smartphone app to make it highly accessible and a point-of-care technique. The challenge related to the LAMP method is the optimization of the primer and reaction conditions.

Rapid diagnostic test (TDR):

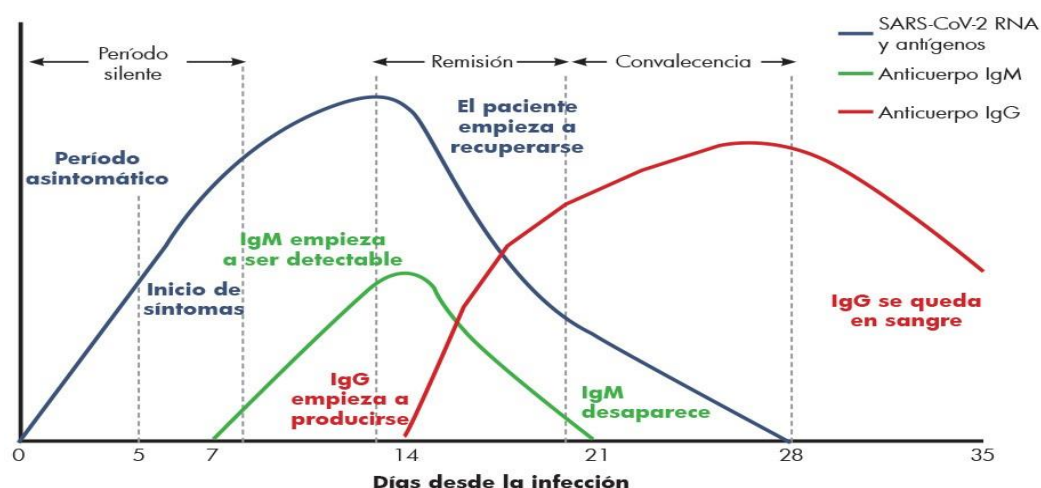
In the case of rapid diagnostic tests, so called because they are able to determine if a person is infected with Covid-19 in about 15 minutes, they have a different system than PCR. These tests do not identify the RNA of the virus, but detect antigens or antibodies produced against the virus using a blood sample, which is another way to know if the patient is or has been infected, or proteins of the virus present in respiratory samples of nasopharyngeal exudate. In this way, and similar to pregnancy tests, a sample of saliva, nostrils or blood is extracted, the latter being the least common. They are applied to a device containing a paper-based immune chromatography, that is, a platform that has the proteins of the virus 'glued' together to detect antibodies specific to the proteins of the virus. Thus, it dictates whether the patient is infected or not. This type of test greatly facilitates the work of health workers, who are currently so overwhelmed, since they can be done at home and frees them to focus on patients and their pathologies, streamlining the country's health system.

Antigen detection tests (rapid Ag. Viral tests):

The viral particle of coronaviruses consists of a nucleocapsid formed by the viral RNA genome associated with nucleocapsid (N) proteins surrounded by an envelope composed of the viral proteins spike (S), envelope (E) and membrane (M). Antigen (Ag) detection tests are based on the detection of SARS-CoV-2-specific viral proteins in the sample, such as the N protein and the S1 or S2 subunits of the spike protein. The sample is obtained from the respiratory tract, usually nasopharyngeal exudate or pharyngeal gold, by a swab, or sputum. The antigen test does not require any additional instruments or equipment, making it highly portable and can be used in a wide variety of healthcare settings at so-called point-of-care and screening. This test allows great benefits to emergency services, which would allow rapid identification of outbreaks and contain the spread of infections. In contrast, the PCR test looks for the presence of genetic material from the virus. However, "the rapid test does not replace PCR, but it is a very good alternative to the need for rapid diagnosis or when PCR diagnoses are not available. Viral antigen or LFT tests detect protein material on the surface of the virus and are very likely to give a positive result when someone is in an active infectious period, while PCR tests detect the genetic material of the virus, which may be present for several weeks after a person is no longer infectious. There is a spectrum of infectious amounts of the COVID-19 virus and we show that LFTs are likely to detect cases 90-95% of the time when people are at their most infectious. The tests could reach 100% sensitivity when viral loads are at their peak and will therefore detect almost everyone who currently poses a serious public health risk. Most likely, if someone's LFT is negative but their PCR is positive, this is because they are not in the maximum stage of transmission.

Antibody detection techniques (IgM/IgG):

They detect the presence of IgM and IgG antibodies against SARS-CoV-2 in a blood, serum or plasma sample. They are mainly used to monitor the course of infection. There are TDRs that detect total antibodies and others that differentiate between IgM and IgG, and can detect IgG or IgM alone or both in the same kit. TDRs are performed on a capillary blood sample obtained from the patient's finger. Serological tests are highly specific but heterogeneous in their sensitivity for the diagnosis of COVID-19. For certain indications, including late disease presentations, serological testing may have added value. The presence of antibodies to SARS-CoV-2 may indicate recent or past COVID-19 infection. Lateral flow immunoassay (LFIA) antibody tests have the advantage of being easy and quick to perform, but many have low sensitivity in acute settings. The enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassays (CLIA) have higher sensitivities. In addition to humoral immunity, cellular immunity is also essential for the success of host defenses against viruses. Enzyme-linked immunospot assays (ELISpot) can be used to measure T-cell responses against SARS-CoV-2. The presence of cross-reactive SARS-CoV-2-specific T cells in never-exposed patients suggests the possibility of cellular immunity induced by other circulating coronaviruses. T-cell responses against SARS-CoV-2 have also been detected in patients recovered from COVID-19 without detectable antibodies. The following illustrative figure represents the kinetics of Covid-19 and indicates in a schematic way what types of test is convenient according to the period in which the patient is.



Conclusion:

During a pandemic, the only way to tackle the pathogen is to limit its spread, which is only possible if affected people are detected and separated as soon as possible. The identification of SARS-CoV-2 in Wuhan, China, using sequencing techniques, was a breakthrough because its identification led scientists to advance its diagnostic and therapeutic studies. The first technique recommended for its diagnosis by the China CDC was RT-PCR. This technique was and continues to be widely used during the current COVID-19 pandemic. It is the same technique that was used to diagnose SARS-CoV in 2002. Lessons learned from the SARS-CoV outbreak have guided the very early identification of SARS-CoV-2 infection using sequencing and RT-PCR techniques. During the course of time, since its identification, there has been an immense study on the development of rapid nucleic acid-based tests to detect COVID-19 disease, among which it is important to mention SHERLOCK, CRISPR and other nucleic acid-based diagnostic kits. Side flow. These latest diagnostic approaches parallelly compete in diagnostic accuracy with RT-PCR based diagnosis, however, biosensor related diagnostics need further understanding and optimization to fit pathogen diagnosis without the need for further confirmation using biosensor based tests. RT-PCR. Another approach was the establishment of serology-based diagnostic tests that are comparatively easy to handle and do not need sophisticated machines or trained personnel like RT-PCR and can be easily used in home settings to decrease the exposure of health professionals who are in high risk of contracting an infection during this pandemic. This manuscript has tried to bring to light various diagnostic approaches based on serology as shown in that it is based on IgG/IgM antibodies. The asymptomatic spread of COVID-19, as reported by some research groups, made it crucial to develop multiplex and point-of-care techniques such as isothermal amplification, so that they can be used to test the majority of the population and isolate infected people. Mainly in remote areas, quarantine centers, in developing countries that lack sufficient resources and skills. Data on COVID-19 and its variants is evolving very rapidly, and there is no doubt that some of the details in this article may change as more studies become available. This review will help the reader understand established techniques and other promising techniques in the management of pandemic diseases such as COVID-19.

Conflict of interest:

The Author declares that there is no conflict of interest with this manuscript.

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