

Diagnostic tests for COVID-19 detection: A hybrid methodology

Pruebas de diagnóstico para detectar la COVID-19: una metodología híbrida

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Clinical diagnosis is a requirement for proper treatment to be started in everyday medical practice. Treatment implementation is varied: observation, medication prescription, surgical procedures initiation, prophylaxis for the future complications, prevention of inappropriate medication combinations and, in current times, isolation or hospitalization of patients with coronavirus disease 2019 (COVID-19).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a type of human coronavirus of zoonotic origin made up of single-stranded RNA and is the causative agent of current COVID-19 pandemic. The spectrum of the disease is broad: it can be asymptomatic, produce mild symptoms, or cause acute respiratory disease or severe pneumonia. At present, there is no officially approved specific treatment or vaccine.

If the presence of COVID-19 virus is detected in asymptomatic people, they could be isolated and thereby possibly prevent spreading the disease to people at high risk of complications, such as older adults and individuals with immune deficiencies. It should be noted that these are the groups that are most affected by seasonal influenza, which contrasts with the 2009 A H1N1 pandemic, which severely

affected people younger than 65 years and without complications¹.

To define screening tests diagnostic accuracy, epidemiology of the disease has to be known. Internet online reports, such as the Johns Hopkins Coronavirus Resource Center COVID-19 tracking page², indicate that, by May 16, 2020, 45,032 confirmed cases had been recorded in Mexico, and among them, 4767 deaths. It should be considered that there is a delay in the transmission of data from hospitals to the electronic network. The case-fatality rate was calculated at 10.6%, a figure that contrasts with 4.1% in California, 6.7% in Boston or 7.9% in New York³.

Reviewing COVID-19 national monitoring by the Institute of Geological and Atmospheric Research⁴, the sentinel model estimate is 394,257 cases, out of which 51,229 have been confirmed and 5703 people have died (on May 17, in comparison with the figures reported by Johns Hopkins). We obtained data indicating that 163,691 people have undergone some test in Mexico³. There is also information from other countries, and specifically in US States where a high percentage of Mexican-Americans live, such as Texas, 678,471 tests have been carried out, among which 46,999 are positive cases, and mortality is also

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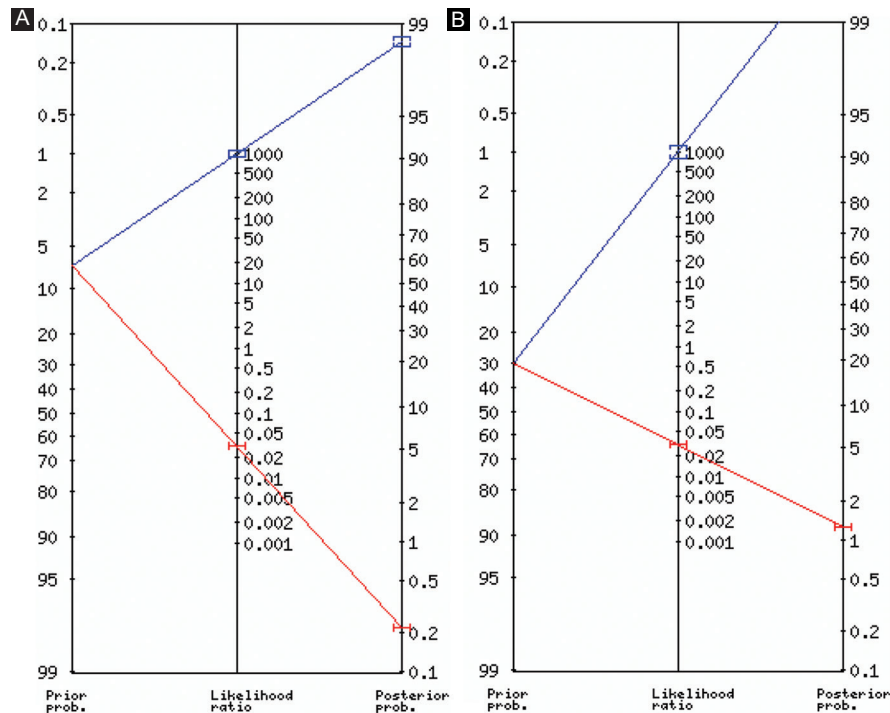


Figure 1. Fagan's nomogram to calculate the probabilities before and after the test. Texas data. **A:** Likelihood is 0.069 before the test, with a sensitivity of 0.97 and specificity of 0.999 being considered. **B:** Pre-test likelihood of having COVID-19 in Mexico is 0.3, with the same diagnostic sensitivity and specificity. The upper diagonal line of the nomogram is LR+ and the lower one is LR-. Post-test likelihood is shown with its 95% confidence interval. (Diagnostic test calculator, version 2010042101: <http://araw.mede.uic.edu/cgi-bin/testcalc.pl>).

reported in 1305 cases. Patient age mode is between 40 and 49 years, but the highest mortality occurs in people older than 80 years, although it affects all ages to a lesser extent.

In Texas, approximately 6.9% of people who have undergone a test have been positive, and 2.8% of infected people have been reported to have died. These calculations are still highly inaccurate, but they can be useful despite not knowing what the symptoms were and the circumstances diagnostic tests were indicated in.

What are all these numbers useful for? They help to calculate pretest likelihood. In Texas, tests are made to suspicious patients, but are also carried out by private laboratories that report their cases to the State. Then, the likelihood of suffering from COVID-19 before the test is 0.069, and considering that real-time polymerase chain reaction (RT-PCR) has a sensitivity of 97% and specificity of 100%, it does not mean that diagnosis is 100% correct. Positive likelihood ratio ($LR+ = \text{sensitivity} / [1 - \text{specificity}]$) and negative likelihood ratio ($LR- = [1 - \text{sensitivity}] / \text{specificity}$) can be calculated. If we reasonably consider that specificity is 0.999, for each sample of 100,000

individuals, we will have 6900 sick subjects, out of whom 207 will be false negatives, and for the rest, 93,100 people without infection, we will have 93 false positives (calculated with Fagan's nomogram⁵, figure 1, left panel). These false positives may correspond to technical problems, contamination, or presence of viral remains in the mucosa. Positive tests have been reported in people who had a history of having been COVID-19-positive cases and had a "reinfection". However, so far, these individuals have not been shown to be likely to infect⁶, and neither has it been possible for reinfection to be obtained in non-human primate models⁷.

Defining who should be tested for COVID-19

Symptoms such as fever, cough, or slight shortness of breath on anamnesis, and close contact with a person who is known to have COVID-19 or has recently traveled from a contaminated area, are indications to stay home and seek medical attention. Older patients and people who have serious underlying medical conditions or are

immunocompromised should get in touch with their health-care provider immediately, even if symptoms are mild. If somebody has severe symptoms, such as persistent chest pain or tightness, confusion, impaired alertness, inability to wake up, or bluish lips, they should immediately contact and attend a clinic or emergency room at some hospital and seek medical attention. Trained health personnel will determine if a confirmatory diagnostic test should be carried out, and if so, a nasal swab or also a saliva (sputum) sample will be obtained for laboratory analysis.

If the tests are carried out in patients with the described characteristics, pre-test likelihood increases. Let's accept that the likelihood of having COVID-19 if the test is indicated in Mexico is 0.31 (4.5 times higher than that calculated in Texas), and let's consider that the test is only performed in cases with a high level of suspicion; this way, maintaining the diagnostic yield that was calculated for Texas, out of every 100,000 tested individuals we would have 30,000 sick subjects, and among them, 900 would be false negatives. As for the 70,000 negative cases, we might have only 70 false positives. The probability of having COVID-19 if the test was positive is 99.9% (Fig. 1B).

Molecular platforms to specifically diagnose COVID-19

PCR is a molecular technique that allows copies of a particular DNA region to be generated *in vitro*. PCR depends on a thermostable enzyme (DNA polymerase), Taq polymerase, and requires DNA primers specifically designed for the DNA region of interest. The results are obtained in a few hours, and the test has enough specificity to differentiate the COVID-19 virus from other viruses that cause similar symptoms⁸.

The PCR technique offers three useful characteristics in pandemic situations: it is specific, it can differentiate between two evolutionarily extremely close microorganisms; it is sensitive, it detects amounts as low as 15 copies and even less of viral genetic material; and finally, it is an anticipatory technique, that is, the virus can be detected at early stages of respiratory complications. However, the need arises to consider faster diagnostic alternatives given the probability of the appearance of genetic variants in the virus evolution.

Serological platforms for COVID-19 rapid diagnosis

Considering the “technical details” the PCR technique entails, rapid diagnostic tests that use a drop of blood from a finger are currently available. These tests allow to know in < 10 min if a person is infected or not. Rapid serological tests measure the development of immunoglobulins (Ig) such as those of the IgM class, which are the first antibodies to be formed in the presence of infection; their concentration is not too high and they are short lived, which is why their detection indicates acute infection. One exception is that IgM can measure activity of a disease at chronic stages (as in hepatitis B).

Specific IgG-class antibodies are the most abundant in our body, but they can take some time to be generated after an infection or vaccination. IgG concentration increases until, 3-6 weeks after infection, it reaches a plateau that very slowly descends. Its persistence is usually prolonged, far beyond the healing of the patient, and sometimes it is detectable throughout life.

The detection of specific IgM at certain concentrations, given the briefness of its duration, enables us to establish a “probable” diagnosis of acute infection. On the other hand, the observation of an increase in the concentration of a specific IgG in two samples separated in time (one in the acute phase and the other in the convalescence phase, usually 2 weeks apart) indicates the presence of an antigenic stimulus at that moment, or what is the same, existence of an acute infection⁹. In addition, they can be useful for analyzing immunity persistence.

Nanostructured platforms for COVID-19 virus long-term qualitative identification

In recent years, Raman spectroscopy has been used for rapid identification of unknown materials, such as high-purity chemicals analysis, measurement of pharmaceutical ingredients, or polymer characterization. Nanostructured platform instrumentation employs an intelligent software for decision-making and is aided by integrated spectral libraries, which makes of it an ideal technique for molecular analysis purposes.

This spectroscopy uses the scattering of light emitted by a monochromatic laser to explore the molecular structure of matter. For every million scattered photons, a single photon will interact with the vibratory

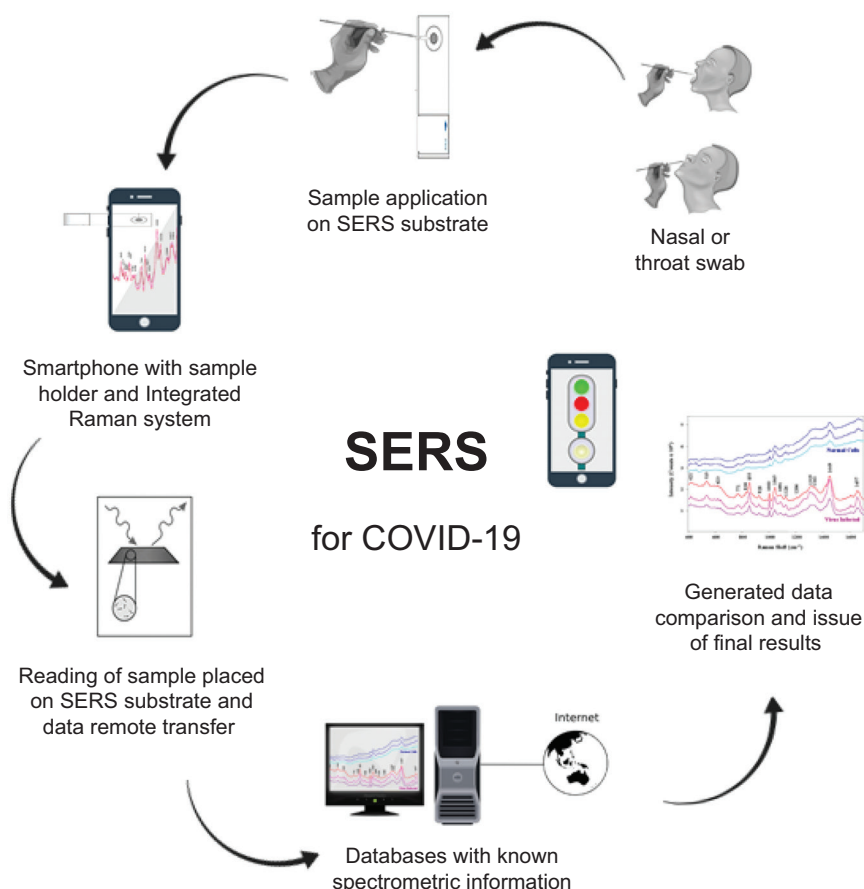


Figure 2. Raman spectroscopy methodology for COVID-19.

states of a molecule of the analyzed sample and emit light with a different wavelength. In turn, the surface-enhanced Raman scattering technique (Fig. 2) increases sensitivity and specificity, which makes of it a technique with analytical applications in infectious diseases such as COVID-19.

Advances in the field of component miniaturization have led to the design of manually-operated, portable, and high-performance Raman devices. The sample analysis procedure involves calibration of the device for correcting inconsistencies associated with handling errors by operators of the device. The methodologies thus created are reliable for unequivocal identification of materials. Scanning and decision-making process total time takes approximately 20 s until “approval/disapproval” of the material or molecule in question is obtained¹⁰.

On May 8, 2020, the US Food and Drug Administration authorized the first diagnostic test for COVID-19 that uses a nasal sample to be collected at home. It

consists of a laboratory-developed test of the molecular type with high specificity, developed by the Rutgers Clinical Genomics Laboratory. Authorization of additional diagnostic tests with the option of home sample collection will continue to increase patient access to COVID-19 testing. This provides an additional option for an easy, safe, and convenient collection of the samples that are required for testing, without having to attend a medical practice, hospital or laboratory.

Epilogue

The approach to diagnostic utility analysis is carried out from a Bayesian perspective, in which the test result is known and the likelihood of having the disease is analyzed. To that end, internal diagnostic accuracy indices, such as sensitivity and specificity, should be analyzed as soon as possible, given that, applied to disease epidemiology, they play an

important role for defining the target population the diagnostic test is directed to, as well as the likelihood of disease and false negatives after the test. Carrying out an indiscriminate screening in the general population is clearly not advisable, given that public money will be wasted. The presence of false negatives will increase as more tests are performed. Knowing and applying these indices will help to efficiently use public resources.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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