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Abstract

Latin America accounts for roughly one-quarter of global COVID-19 cases and one-third of deaths. Health inequalities in the region lead to barriers regarding the best use of diagnostic tests during the pandemic. There is a need for a simplified guideline in the region that takes into consideration the available health resources, international guidelines, medical literature, and local expertise.

Nine experts from different Latin American countries developed a simplified algorithm for COVID-19 diagnosis in the region, using a modified Delphi method. Twenty-four questions related to diverse diagnostic settings were initially proposed, followed by an extensive discussion of the literature and experts' experience.

According to time from close contact or symptom onset, the algorithm considers three different timeframes ([?]7 days, 8–13 days, and [?]14 days) and discusses diagnostic options for each one. SARS-CoV-2 real-time reverse transcription polymerase chain reaction (rRT-PCR) continues to be the diagnostic test of choice from Day 1 to Day 14 after symptom onset or close contact, although antigen testing may be used in high-prevalence settings or in particular situations (such as from Day 0 to Days 5–7 of symptom onset). Antibody assays may be used for diagnostic confirmation, mainly after Day 14, if there is an epidemiological or individual need. If the clinical suspicion is very high, but other tests are negative, these assays may be used as an adjunct to decision-making from Days 8–13.

The proposed algorithm is intended to be used as a support for COVID-19 diagnosis decision-making in Latin America.

Introduction

In May 2020, the Pan American Health Organization (PAHO) declared the region an epicenter of the disease (Pan American Organization, 2020) and in November 2020, cumulative COVID-19 cases in Latin America accounted for around 24% of all cases and 33% of all deaths globally. (World Health Organization, n.d.)

Although PAHO (Pan American Health Organization, 2020) and other organizations (World Health Organization, 2020a)(2019-nCoV Working Group. Communicable Diseases Network Australia., 2020) have released laboratory guidance for diagnosing COVID-19 cases, few have considered the availability of tests when making these recommendations. Latin America is a region with great socio-economic contrasts (World Bank Development Indicators DataBank, n.d.), as well as unequal health resources and, like in developed countries (Pablos-Méndez et al., 2020), the availability of COVID-19 tests and trained personnel are not exempt from supply chain or personnel pressures.

A panel of Latin American experts gathered to discuss the best use of diagnostic methods in the region and propose a simplified algorithm.

Methods

A modified Delphi method was used to prepare an algorithm using the iADVISE platform (Within3, OH, USA). Nine experts from Latin American countries iteratively answered 24 online questions about diagnostic methods and their application in specific cases for two weeks. The questions were written by an external microbiologist and infectious diseases specialist with high expertise in the area and reviewed by a multidisciplinary panel. The experts also met to review the proposed algorithm in two online meetings during this period.

The consensus level was determined for each of the 24 initial questions using a simple yes/no count. For questions with a low level of agreement (less than 7/9 matched responses), further discussion was necessary to reach a consensus. Recommendations were only made if the consensus level was above this threshold.

Consensus results

The proposed algorithm is divided into three parts according to time after first contact or time after symptom onset (Fig. 1).

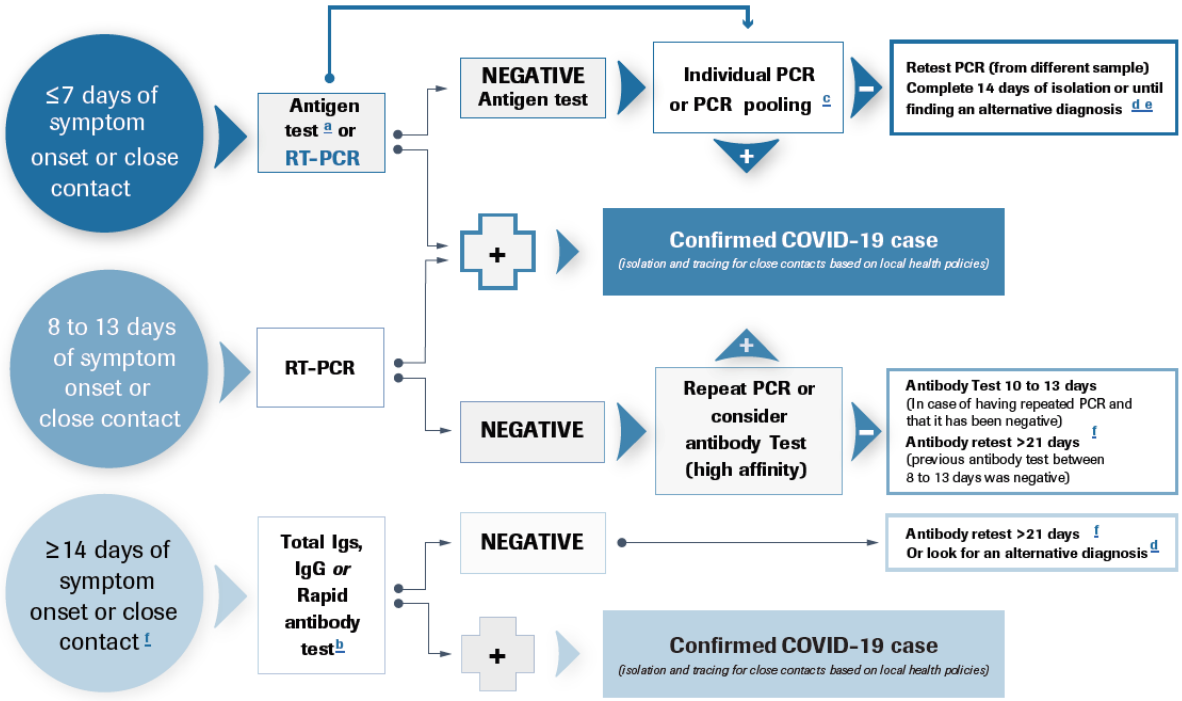
The rRT-PCR is recommended as the primary test in the initial 14 days after symptom onset or close contact. Early sample collection from the upper respiratory tract minimizes the probability of negative rRT-PCR test results (Mallett et al., 2020). If negative, rRT-PCR may be repeated in a different sample, at the discretion of the physician. Antigen detection tests are most likely to perform well in patients with high viral loads in the pre-symptomatic (1–3 days before symptom onset) and early symptomatic phases (within the first 5–7 days of illness)(World Health Organization, 2020). These tests may be used as an alternative in high-prevalence settings or special cases when rRT-PCR tests are unavailable.

The rRT-PCR is the diagnostic test of choice between 8–13 days after initiation of symptoms. If results are negative, rRT-PCR may be repeated. In some cases, antibody assays may be used during this period, but considerations should be taken when the results are negative (false negative tests are still common in this period), or when IgM is positive and IgG is negative, raising the possibility of a false positive test (Deeks et al., 2020).

Fourteen days after symptom onset or close contact, antibody detection assays are recommended as the initial test for the detection of SARS-CoV-2 infection in immunocompetent hosts. In most cases, antibody

assays are used to trace contacts or for other epidemiological reasons(Jayamohan et al., 2020), but may be used for individual diagnosis in specific circumstances.

Figure 1. A simplified alternative diagnostic algorithm for SARS-CoV-2 suspected symptomatic patients and confirmed close contacts (asymptomatic)



[a]Ideal use only in high-prevalence (>5–10%) scenarios with symptomatic patients or selected settings (e.g. emergency rooms, elderly residences, healthcare personnel, and surgical urgencies). The best timeframe for collection in asymptomatic individuals is 5–7 days after the close contact. Providers conducting testing on asymptomatic populations must be aware of the potential for a presumed false-positive result with an antigen test that will necessitate confirmation with a subsequent RT-PCR test (Virginia Department of Health, 2020).

[b]Consider the interpretation of the result as "Confirmed exposure to SARS-CoV-2". In the case of IgM positivity only, consider as a probable false positive (Kubina and Dziedzic, 2020) and repeat determination with other methods, such as high-affinity antibody assays (total immunoglobulins or IgG).

[c]Consider PCR pooling for population screening with low pre-test probability (<10%) to ensure assay cost-effectiveness or in negative antigen patients. If the pooling result is positive, individual RT-PCR must be performed for each pooled sample, so the maximum recommended pool size is 10 (CDC, 2020a).

[d]Consider multiplex RT-PCR, including influenza A/B or respiratory panel with influenza, RSV, and other viral/bacterial/fungal pathogens (Kim et al., 2020) (Zhu et al., 2020). The presence of other respiratory viruses does not rule out co-infection by SARS-CoV-2, therefore this possibility should not be neglected (and should be thoroughly investigated if the clinical-epidemiological context is suggestive of such).

[e]Consider antibody tests if other results are negative.

[f]Consider Day 14 of symptoms or Day 21 of close contact.

Ig, immunoglobulin; PCR, polymerase chain reaction; rRT-PCR, real-time reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RSV, Respiratory Syncytial Virus

Discussion

Available diagnostic methods

Criteria for choosing tests in resource-constrained settings

The World Health Organization (WHO) has published the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) criteria (Kosack et al., 2017) that may be used as a benchmark for identifying the most appropriate diagnostic tests for resource-constrained settings. However, these criteria are non-specific and need to be adapted to each diagnostic need, and not all test methods can be simplified to match the ASSURED criteria.

Kosack et al. also identified six steps that must be additionally addressed when selecting an *in vitro* diagnostic test: (a) define the test's purpose; (b) review the market and check each product's specification; (c) review the test's regulatory approval; (d) obtain data on the diagnostic accuracy of the test under ideal conditions (i.e. in laboratory-based evaluations); (e) obtain data on the diagnostic accuracy of the test in clinical practice; and (f) monitor the test's performance in routine use.

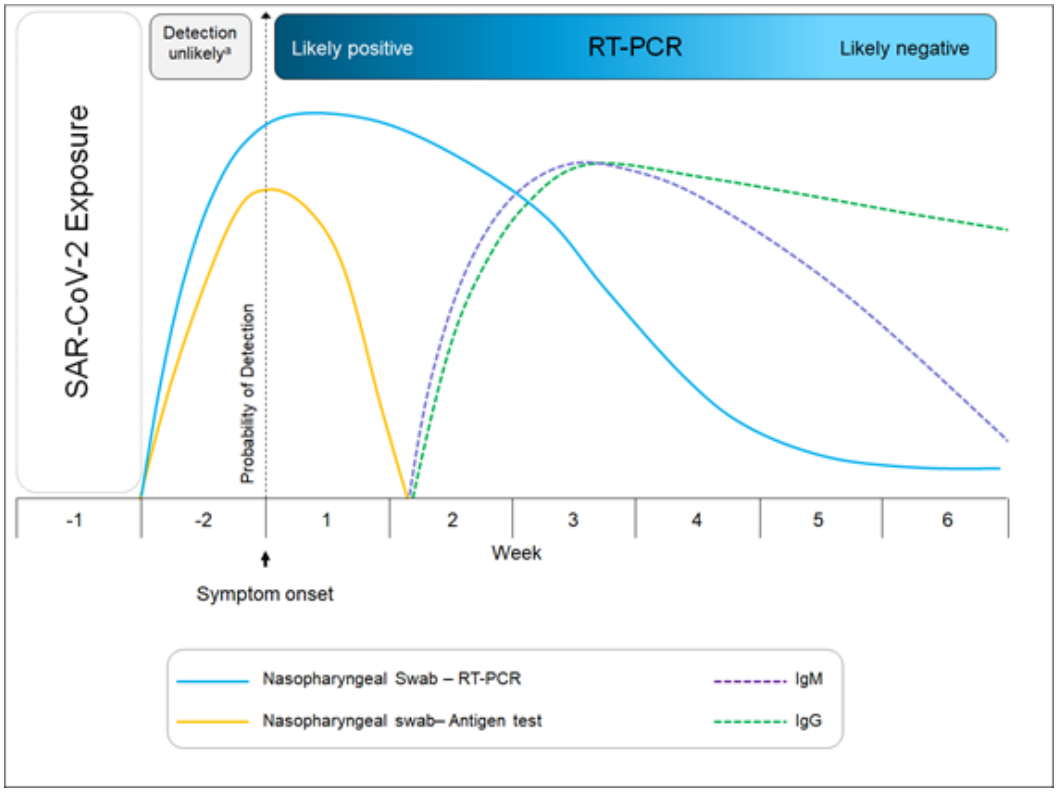
These six steps should all be considered when selecting an *in vitro* diagnostic test for routine use.

Viral detection according to the clinical course

The detection of viral particles (Mallett et al., 2020) (Siam et al., 2020) or the corresponding immunological response (Deeks et al., 2020) varies with post-infection time (Fig. 2). A systematic review concluded that collecting samples early in the course of disease minimizes the risk of false-negative results (Mallett et al., 2020).

Another systematic review of immunological response studies summarized assay results for IgG, IgM, IgA, total antibodies, and IgG/IgM since the onset of symptoms (Sethuraman et al., 2020). All showed low sensitivity during the first week (30.1%, 95% CI 21.4–40.7), which increased in the second week (72.2%, 95% CI 63.5–79.5), and peaked in the third (91.4%, 95% CI 87.0–94.4) and fourth weeks (96.0%, 95% CI 90.6–98.3). Specificity was not evaluated according to time but was generally high for IgM (99.1%, 95% CI 97.5–99.8) and IgG (98.6%, 95% CI 96.7–99.5).

Fig. 2. Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset (modified from Sethuraman *et al* . 2020)



[a]Detection only occurs if patients are followed up proactively from the time of exposure.

Ig, immunoglobulin; PCR, polymerase chain reaction; RT-PCR; real-time reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Real-time reverse transcriptase polymerase chain reaction (rRT-PCR)

Real-time polymerase chain reaction (rRT-PCR) is the gold-standard molecular technique for the detection of SARS-CoV-2 viral RNA in all recommended samples. It targets the following sequences that code for structural viral proteins: spike (S), membrane (M), envelope (E), nucleocapsid (N), and RNA-dependent RNA polymerase (RdRP). Both S and N proteins are highly immunogenic (Ravi et al., 2020). The S proteins seems to be the major target of neutralizing antibodies for correlated coronaviruses (Berry et al., 2010). High infectivity of SARS-CoV-2 has compelled the CDC to publish rRT-PCR primers and probes together with all relevant literature for public access (Khalaf et al., 2020). The positive rate of rRT-PCR detection is dependent on the sample type, with differences between bronchoalveolar lavage fluid (93%), fibrobronchoscopy brush biopsy (46%), sputum (72%), nasal swabs (63%), pharyngeal swabs (32%), feces (29%), and blood (1%) (Wang et al., 2020). Combining nasopharyngeal and oropharyngeal swabs is now one of the most commonly used specimen types for diagnosing COVID-19 active infection (Lai and Lam, 2020). In September 2020, the WHO published a guideline not recommending saliva as the only specimen type for routine clinical diagnostics, because of the wide variation in collection methods (World Health Organization, 2020a).

The virus can be detected at least 48 hours before the onset of symptoms (pre-symptomatic cases), up to 12–14 days (at least 6–7 days) after the onset of symptoms in samples from the upper respiratory tract (NP/OP

swabs), and for a median of 20 days in samples from the lower respiratory tract, including sputum, tracheal aspirate, and bronchoalveolar lavage (Mallett et al., 2020) (World Health Organization, 2020a) (Lippi et al., 2020).

Pooling rRT-PCR samples increases testing efficiency, which may be particularly helpful in areas with low prevalence and few health resources, given that only a limited number of tests are available (He et al., 2020). The idea is to pool samples from several individuals and test the combined sample with a single test. If the test is negative, all subjects are negative. If the test is positive, all individuals must be tested again to find the infected patient(s) (Food and Drug Administration USA, 2020). The US Food and Drug Administration initially proposed that five was the maximum number of samples to be pooled for rRT-PCR, but other studies found that the ideal number of pooled samples depends on the disease prevalence in the tested population (Hanel and Thurner, 2020) (Deckert et al., 2020) (Cherif et al., 2020)· One potential constraint of pooled testing is that the false-negative rate may increase owing to dilution of positive samples, therefore high-sensitivity rRT-PCR tests are adequate to minimize this limitation (Lorentzen et al., 2020). In general, the larger the pool of specimens, the higher the likelihood of generating false-negative results (CDC, 2020a).

As with all diagnostic tests, the predictive value of rRT-PCR depends highly on its specificity, sensitivity, and prevalence of the disease in the target population (CDC, 2020a) (Table 1). False-negative results may also result from technical issues, from sampling to amplification, including thermal inactivation (Siam et al., 2020). A confirmatory test (e.g. repeated rRT-PCR) may be warranted if the initial results are negative, and the clinical characteristics are suggestive of infection (Lai and Lam, 2020) (Lorentzen et al., 2020) .

TABLE 1. Correlation between pre-test probability and test results[a]

[a]Modified from Siam *et al. 2020*

[b]Hellou *et al.* 2020

LAMP, loop-mediated isothermal amplification; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; rRT-PCR, real-time reverse transcription PCR

Antigen detection assay

Another COVID-19 detection method involves the direct detection of SARS-CoV-2 viral particles using immunoassays (Ji et al., 2020). The SARS-CoV-2 nucleocapsid protein may be detected in nasopharyngeal swabs and urine samples of COVID-19 patients within 3 days of onset of fever (Diao et al., 2020).

A Cochrane systematic review found that sensitivity varied considerably across studies (from 0–94%). Based on eight evaluations in five studies on 943 samples, the average sensitivity was 56.2% (95% CI 29.5–79.8) and average specificity was 99.5% (95% CI 98.1–99.9) (Dinnes et al., 2020). Data for individual antigen tests were limited, with no more than two studies for any test. There were no studies in asymptomatic individuals.

For asymptomatic individuals, a non-peer-reviewed study showed that for a pre-test probability of 5%, the negative predictive value was 99.6% (95% CI 99.5–99.7) and the positive predictive value was 81.5% (95% CI 65.0–93.2) (Alemany et al., 2020). At this pre-test probability, the estimated number of false-negative and false-positive values per thousand tests were 4 (95% CI 3–5) and 12 (95% CI 4–27), respectively. The authors stressed the need for confirmatory testing of positive tests with nucleic acid amplification techniques in these circumstances (Alemany et al., 2020).

In comparison with rRT-PCR, rapid antigen detection tests tend to have a lower sensitivity, and owing to the increased risk of false-negative results, some authors consider such tests only as an adjunct to rRT-PCR tests (Siam et al., 2020). Alternatively, antigen detection tests have the advantage of being simple to perform and can play a role in settings where accessibility to rRT-PCR tests is limited or in symptomatic patients

with a high viral load and within the first 5–7 days after symptom onset (Lai and Lam, 2020). The viral load is directly related to the sensitivity of the test (Dinnes et al., 2020).

Antibody assays

Serologic tests are essential because they provide information on patients who have been infected and already recovered, and asymptomatic patients who were never diagnosed (Ravi et al., 2020). In a study that followed the immunological response in COVID-19 patients, three types of seroconversion were observed: synchronous seroconversion of IgG and IgM (nine patients), IgM seroconversion earlier than that of IgG (seven patients), and IgM seroconversion later than that of IgG (ten patients) (Long et al., 2020). A study profiling the early SARS-CoV-2 humoral response found that the median time for IgM detection was five days after symptom onset; IgG was detected at a median of 14 days after symptom onset (Guo et al., 2020).

For SARS-CoV-2, the IgG and IgM produced specific to the S and N proteins are of particular diagnostic interest. A study indicates that the S protein tends to cause a more significant immune response than the N protein, eliciting neutralizing antibodies (Amanat et al., 2020). However, other studies argue that the N protein is more immunogenic, as it is expressed abundantly during active infection (Johns Hopkins Center for Health Security, 2020).

Some examples of serologic tests to measure patient antibodies include rapid diagnostic tests (RDTs), enzyme-linked immunoassays (ELISAs), chemiluminescent immunoassays (CLIAs, not to be confused with the CLIA acronym: Clinical Laboratory Improvement Amendments), and neutralization assays (Ravi et al., 2020), performed only at specialized laboratories. Another review (Deeks et al., 2020) found that some differences were noted by test technology, with CLIA methods appearing more sensitive (97.5%, 95% CI 94.0–99.0) than ELISA (90.7%, 95% CI 83.3–95.0) or colloidal gold immunoassay (CGIA)-based lateral flow assays (90.7%, 95% CI 82.7–95.2) for IgG/IgM (there were also differences in sensitivity for IgG but no differences for IgM). There was little clear evidence of differences in specificity between technology types.

Essential considerations for antibody testing include timing of the test, previous infection, immune status of the individual, and cross-reaction, which can alter the test results (Siam et al., 2020).

CRISPR technology

The CRISPR gene-editing tool has been utilized to construct an accurate, faster, and simple-to-use SARS-CoV-2 detection test. The DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) assay is based on CRISPR–Cas12 and can distinguish SARS-CoV-2 with no cross-reactivity for related coronavirus strains, using N gene genomic RNA, within 40 minutes (Broughton et al., 2020).

Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a method of isothermal DNA replication. It utilizes six DNA oligonucleotides that hybridize with eight different regions of a target molecule in an accelerated format. Reverse transcriptase can be included to improve sensitivity within the reaction when detecting an RNA target (RT-LAMP), such as SARS-CoV-2 RNA (Rabe and Cepko, 2020).

Table 2. Comparison of diagnostic options for SARS-CoV-2 detection[a]

[a]Modified from Siam *et al. 2020*

[b]Hellou *et al.* 2020

LAMP, loop-mediated isothermal amplification; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; rRT-PCR, real-time reverse transcription PCR

Special considerations

rRT-PCR vs. antigen test

rRT-PCR is the recommended initial test for diagnosing SAR-CoV-2 in symptomatic patients in international guidelines (Pan American Health Organization, 2020) (2019-nCoV Working Group. Communicable Diseases Network Australia., 2020) (CDC, 2020c). However, as the number of patients presenting with COVID-19 symptoms increases, there has been a shortage of diagnostic resources, such as swabs, PCR reagents, and RNA isolation kits, and a growing demand for rapid, onsite diagnostics (Ravi et al., 2020).

Point-of-care (POC) tests, including rapid antigen detection tests, are also recommended as an initial test by the CDC (CDC, 2020b), particularly in the early days of symptoms or in cases of close contacts in high-risk congregate settings. Infection prevalence at the time of testing and clinical context both impact pre-test probability (CDC, 2020b) (Table 1) and should be taken into account before and after test results. Testing of asymptomatic contact cases may be considered after 5–7 days of contact, even if the antigen detection tests are not explicitly authorized for this use. Asymptomatic cases have been demonstrated to have viral loads similar to symptomatic cases. A negative antigen detection test should not remove a close contact individual from quarantine requirements (World Health Organization, 2020).

Compared with rRT-PCR, antigen detection tests are cheaper, have a similar specificity, and usually deliver results faster, but have a lower sensitivity (CDC, 2020b). The choice of test also depends on the availability of tests and trained personnel, along with the above factors.

Types and results of immunological tests

Antibody tests available for laboratory use include ELISA, more advanced CLIA, and laboratory-independent POC lateral flow assays for rapid detection of antibodies (such as CGIA), among others (Deeks et al., 2020).

CLIA methods appear more sensitive (97.5%, 95% CI 94.0–99.0) than ELISA (90.7%, 95% CI 83.3–95.0) or CGIA-based lateral flow assays (90.7%, 95% CI 82.7–95.2) for IgG/IgM (Deeks et al., 2020). Tests that detect antibodies with a high affinity for the SARS-CoV-2 virus are more likely to indicate neutralizing antibodies (Jayamohan et al., 2020).

Negative rRT-PCR or antigen detection tests and the need for quarantine

In the case that an individual was only in close contact with an infected patient and has not developed symptoms, they should complete 14 days of isolation. No tests are necessary, except for particular cases such as hospitalized patients or for other epidemiological reasons (World Health Organization, 2020b).

Image studies

Chest computed tomography (CT) is considered the primary imaging diagnostic modality for examining patients with COVID-19 (Güneyli et al., 2020). A Cochrane review of radiologic tests (Salameh et al., 2020) showed that the pooled sensitivity of CT was 86.2% (95% CI 71.9–93.8 [13 studies, 2,346 participants]) and specificity was 18.1% (95% CI 3.71–55.8). In patients with negative RT-PCR tests, Ai *et al.* (Ai et al., 2020) suggested that a combination of exposure history, clinical symptoms, typical CT imaging features, and chest CT dynamic changes should be used to identify COVID-19 cases.

Conclusions

COVID-19 diagnosis is always a cause for uncertainty among physicians, health professionals, and public health authorities. Our methodology, involving 24 questions answered by Latin American experts, resulted in a simplified algorithm involving symptomatic people or close contacts in 3 windows of time ([?]7 days, 8–13 days, and [?]14 days).

Even considering the high disparities in healthcare access within the region, we regard rRT-PCR tests as the standard diagnostic tests for SARS-CoV-2 infection, from the onset of symptoms until 13 days afterward. This recommendation is consistent with all main published guidelines (Pan American Health Organization, 2020) (World Health Organization, 2020a). Sample pooling should be used in low-resource and low-prevalence (<30%) settings (CDC, 2020a).

We also recommend tests for antigen detection from upper respiratory tract samples as a simple POC diagnostic test in high-prevalence settings up to 5–7 days after onset of symptoms (World Health Organization, 2020). We consider antigen testing a good alternative to rRT-PCR in this setting, particularly where the molecular tests are not readily available. Antigen detection tests also appear as a reasonable option in the CDC guidelines for SARS-CoV-2 detection (CDC, 2020c).

Immunologic assays are not ideal for diagnosis in the early days of SARS-CoV-2 infection according to WHO and PAHO guidelines (World Health Organization, 2020a) (Pan American Health Organization, 2020). However, we suggest that they can be used from 14 days after symptom onset or 21 days after close contact, if asymptomatic, for epidemiological and contact tracing purposes. Or in some exceptional situations when the other direct diagnostic tests are repeatedly negative in a patient with high clinical suspicion and the individual diagnosis is necessary before that period above mentioned, we recommend the use of antibody assay from Day 8 onward as an aid to the diagnosis.

Depending on local epidemiology and clinical symptoms, for all suspected COVID-19 patients, diagnostic testing for other conditions such as malaria, dengue, typhoid, influenza, and other respiratory diseases should also be considered (Chi et al., 2020) (United Nations. Department of Healthcare Management and Occupational Safety and Health, 2020).

In summary, the proposed simplified algorithm aims to support medical decision-making in Latin America, taking into account published international guidelines and the region's health access inequalities.

Limitations

Although based on well-established consensus formation techniques and drawing on panelists' expertise, these recommendations do not constitute a statement from the institutions or associations to which these professionals are affiliated. The main limitations of this expert panel consensus are selection bias, observer bias, confirmation bias, publication bias, and cohort effects (different features and pace of the COVID-19 pandemic in each country of Latin America).

Implications

The proposed algorithm may support COVID-19 diagnosis decision-making in Latin America, taking into account published international guidelines and the region's health access inequalities.

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References

2019-nCoV Working Group. Communicable Diseases Network Australia. Coronavirus Disease 2019 (COVID-19) CDNA National Guidelines for Public Health Units 2020. https://www1.health.gov.au/internet/main/publishing.nsf/Content/7A8654A8CB144F5FCA2584F8001F91E2/$File/COVID-19-SoNG-v3.10.pdf (accessed November 8, 2020).

Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology 2020. https://doi.org/10.1148/radiol.2020200642.

Alemany A, Baro B, Ouchi D, Ubals M, Corbacho-Monne M, Vergara-Alert J, et al. Analytical and Clinical Performance of the Panbio COVID-19 Antigen-Detecting Rapid Diagnostic Test. MedRxiv 2020:2020.10.30.20223198. https://doi.org/10.1101/2020.10.30.20223198.

Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 2020;26:1033–6. https://doi.org/10.1038/s41591-020-0913-5.

Berry JD, Hay K, Rini JM, Yu M, Wang L, Plummer FA, et al. Neutralizing epitopes of the SARS-CoV S-protein cluster independent of repertoire, antigen structure or mAb technology. MAbs 2010;2:53–66.

Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol 2020;38:870–4. https://doi.org/10.1038/s41587-020-0513-4.

CDC. Interim Guidance for Use of Pooling Procedures in SARS-CoV-2 Diagnostic, Screening, and Surveillance Testing. Cent Dis Control Prev 2020a. https://www.cdc.gov/coronavirus/2019-ncov/lab/pooling-procedures.html (accessed November 10, 2020).

CDC. Information for Laboratories about Coronavirus (COVID-19). Cent Dis Control Prev 2020b. https://www.cdc.gov/coronavirus/2019-ncov/lab/pooling-procedures.html (accessed November 18, 2020).

CDC. Coronavirus Disease 2019 (COVID-19). Cent Dis Control Prev 2020c. https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html (accessed November 18, 2020).

Cherif A, Grobe N, Wang X, Kotanko P. Simulation of Pool Testing to Identify Patients With Coronavirus Disease 2019 Under Conditions of Limited Test Availability. JAMA Netw Open 2020;3. https://doi.org/10.1001/jamanetworkopen.2020.13075.

Chi Q, Dai X, Jiang X, Zhu L, Du J, Chen Y, et al. Differential diagnosis for suspected cases of coronavirus disease 2019: a retrospective study. BMC Infect Dis 2020;20:679. https://doi.org/10.1186/s12879-020-05383-y.

Deckert A, Barnighausen T, Kyei NN. Simulation of pooled-sample analysis strategies for COVID-19 mass testing. Bull World Health Organ 2020;98:590–8. https://doi.org/10.2471/BLT.20.257188.

Deeks J, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 2020. https://doi.org/10.1002/14651858.CD013652.

Diao B, Wen K, Chen Jian, Liu Y, Yuan Z, Han C, et al. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. MedRxiv 2020:2020.03.07.20032524. https://doi.org/10.1101/2020.03.07.20032524.

Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev 2020. https://www.readcube.com/articles/10.1002%2F14651858.CD013705 (accessed November 12, 2020).

Food and Drug Administration USA. Coronavirus (COVID-19) Update? FDA Authorizes First Diagnostic Test for Screening of People Without Known or [7256].pdf 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-diagnostic-test-screening-people-without-known-or.

Guneyli S, Atceken Z, Doğan H, Altınmakas E, Atasoy KÇ. Radiological approach to COVID-19 pneumonia with an emphasis on chest CT. Diagn Interv Radiol 2020;26:323–32. https://doi.org/10.5152/dir.2020.20260.

Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis 2020;71:778–85. https://doi.org/10.1093/cid/ciaa310.

Hanel R, Thurner S. Boosting test-efficiency by pooled testing for SARS-CoV-2—Formula for optimal pool size. PLOS ONE 2020;15:e0240652. https://doi.org/10.1371/journal.pone.0240652.

He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 2020;26:672–5. https://doi.org/10.1038/s41591-020-0869-5.

Hellou MM, Górska A, Mazzaferri F, Cremonini E, Gentilotti E, De Nardo P, et al. Nucleic-acid-amplification tests from respiratory samples for the diagnosis of coronavirus infections: systematic review and meta-analysis. Clin Microbiol Infect 2020. https://doi.org/10.1016/j.cmi.2020.11.002.

Jayamohan H, Lambert CJ, Sant HJ, Jafek A, Patel D, Feng H, et al. SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial response with associated advantages and limitations. Anal Bioanal Chem 2020:1–23. https://doi.org/10.1007/s00216-020-02958-1.

Ji T, Liu Z, Wang G, Guo X, Akbar khan S, Lai C, et al. Detection of COVID-19: A review of the current literature and future perspectives. Biosens Bioelectron 2020;166:112455. https://doi.org/10.1016/j.bios.2020.112455.

Johns Hopkins Center for Health Security. Comparison of National RT-PCR Primers, Probes, and Protocols for SARS-CoV-2 Diagnostics 2020. https://www.centerforhealthsecurity.org/resources/COVID-19/COVID-19-fact-sheets/200410-RT-PCR.pdf (accessed December 9, 2020).

Khalaf K, Papp N, Chou JT-T, Hana D, Mackiewicz A, Kaczmarek M. SARS-CoV-2: Pathogenesis, and Advancements in Diagnostics and Treatment. Front Immunol 2020;11. https://doi.org/10.3389/fimmu.2020.570927.

Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens. JAMA 2020;323:2085–6. https://doi.org/10.1001/jama.2020.6266.

Kosack CS, Page A-L, Klatser PR. A guide to aid the selection of diagnostic tests. Bull World Health Organ 2017;95:639–45. https://doi.org/10.2471/BLT.16.187468.

Kubina R, Dziedzic A. Molecular and Serological Tests for COVID-19. A Comparative Review of SARS-CoV-2 Coronavirus Laboratory and Point-of-Care Diagnostics. Diagnostics 2020;10. https://doi.org/10.3390/diagnostics10060434.

Lai CKC, Lam W. Laboratory testing for the diagnosis of COVID-19. Biochem Biophys Res Commun 2020. https://doi.org/10.1016/j.bbrc.2020.10.069.

Lippi G, Simundic A-M, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med CCLM 2020;58:1070–6. https://doi.org/10.1515/cclm-2020-0285.

Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–8. https://doi.org/10.1038/s41591-020-0897-1.

Lorentzen HF, Schmidt SA, Sandholdt H, Benfield T. Estimation of the diagnostic accuracy of real-time reverse transcription quantitative polymerase chain reaction for SARS-CoV-2 using re-analysis of published data. Dan Med J 2020;67.

Mallett S, Allen AJ, Graziadio S, Taylor SA, Sakai NS, Green K, et al. At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. BMC Med 2020;18:346. https://doi.org/10.1186/s12916-020-01810-8.

Pablos-Méndez A, Vega J, Aranguren FP, Tabish H, Raviglione MC. Covid-19 in Latin America. BMJ 2020;370. https://doi.org/10.1136/bmj.m2939.

Pan American Health Organization. Laboratory Guidelines for the Detection and Diagnosis of COVID-19 Virus Infection - PAHO/WHO | Pan American Health Organization 2020. http://www.paho.org/en/documents/laboratory-guidelines-detection-and-diagnosis-covid-19-virus-infection (accessed November 9, 2020).

Pan American Organization. PAHO Director says fight against COVID-19 pandemic must include chronic disease care - PAHO/WHO | Pan American Health Organization 2020. http://www.paho.org/en/news/26-5-2020-paho-director-says-fight-against-covid-19-pandemic-must-include-chronic-disease-care (accessed November 8, 2020).

Rabe BA, Cepko C. SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification. Proc Natl Acad Sci U S A 2020;117:24450–8. https://doi.org/10.1073/pnas.2011221117.

Ravi N, Cortade DL, Ng E, Wang SX. Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. Biosens Bioelectron 2020;165:112454. https://doi.org/10.1016/j.bios.2020.112454.

Salameh J-P, Leeflang MM, Hooft L, Islam N, McGrath TA, Pol CB, et al. Thoracic imaging tests for the diagnosis of COVID-19. Cochrane Database Syst Rev 2020. https://doi.org/10.1002/14651858.CD013639.pub2.

Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA J Am Med Assoc 2020;323:2249.

Siam MdHB, Nishat NH, Ahmed A, Hossain MS. Stopping the COVID-19 Pandemic: A Review on the Advances of Diagnosis, Treatment, and Control Measures. J Pathog 2020;2020. https://doi.org/10.1155/2020/9121429.

United Nations. Department of Healthcare Management and Occupational Safety and Health. COVID-19: Differential Diagnosis and Co-infections 2020. https://www.un.org/sites/un2.un.org/files/coronavirus_-guidancecoinfections.pdf (accessed November 22, 2020).

Virginia Department of Health. Antigen Testing Recommendations – Coronavirus 2020. https://www.vdh.virginia.gov/coronavirus/antigen-testing-recommendations/, https://www.vdh.virginia.gov/coronavirus/antigen-testing-recommendations/ (accessed November 18, 2020).

Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 2020. https://doi.org/10.1001/jama.2020.3786.

World Bank Development Indicators DataBank. n.d. https://databank.worldbank.org/reports.aspx?source=2&series=SI.PO
(accessed November 8, 2020).

World Health Organization. Diagnostic testing for SARS-CoV-2 2020a. https://www.who.int/publications-detail-redirect/diagnostic-testing-for-sars-cov-2 (accessed November 8, 2020).

World Health Organization. Considerations for quarantine of contacts of COVID-19 cases. WHO Interim Guidance. 2020b. https://apps.who.int/iris/bitstream/handle/10665/333901/WHO-2019-nCoV-IHR_Quarantine-2020.3-eng.pdf?sequence=1&isAllowed=y (accessed November 13, 2020).

World Health Organization. WHO COVID-19 Explorer n.d. https://worldhealthorg.shinyapps.io/covid/ (accessed November 8, 2020).

World Health Organization (ultimo). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid mmunoassays - Interim guidance 2020. https://apps.who.int/iris/bitstream/handle/10665/334253/WHO-2019-nCoV-Antigen_Detection-2020.1-eng.pdf?sequence=1&isAllowed=y (accessed November 17, 2020).

Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, et al. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res 2020;285:198005. https://doi.org/10.1016/j.virusres.2020.198005.