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Narrative review

Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results

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ABSTRACT

Background: To face the current COVID-19 pandemic, diagnostic tools are essential. It is recommended to use real-time RT-PCR for RNA viruses in order (a) to perform a rapid and accurate diagnostic, (b) to guide patient care and management and (c) to guide epidemiological strategies. Further studies are warranted to define the role of serological diagnosis and a possible correlation between serological response and prognosis.

Objectives: The aim was to guide clinical microbiologists in the use of these diagnostic tests and clinicians in the interpretation of their results.

Sources: A search of literature was performed through PubMed and Google Scholar using the keywords SARS-CoV-2, SARS-CoV-2 molecular diagnosis, SARS-CoV-2 immune response, SARS-CoV-2 serology/antibody testing, coronavirus diagnosis.

Content: The present review discusses performances, limitations and use of current and future diagnostic tests for SARS-CoV-2.

Implications: Real-time RT-PCR remains the reference method for diagnosis of SARS-CoV-2 infection. On the other hand, notwithstanding its varying sensitivity according to the time of infection, serology represents a valid asset (a) to try to solve possible discrepancies between a highly suggestive clinical and radiological presentation and negative RT-PCR, (b) to solve discrepancies between different PCR assays and (c) for epidemiological purposes. **G. Caruana, Clin Microbiol Infect 2020;26:1178**

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Introduction

In December 2019, numerous cases of pneumonia of unknown aetiology were reported in Wuhan (China) [1]. In January, the novel causative virus named SARS-CoV-2 was identified, which spread to other Chinese regions and to other countries, causing a world pandemic [2,3]. The clinical presentation of this disease, named coronavirus disease 2019 (COVID-19), varied from asymptomatic or mild flu-like symptoms to severe bilateral pneumonia with acute respiratory distress and death. A rapid replication of the virus

within the first 24 hr from the infection and the relatively high (about 3) reproduction number were described [4].

The available viral genome sequences allowed to soon recognize the close relationship between SARS-CoV-2 and SARS-CoV-1, the causative pathogen of the 2002–2004 outbreak, presenting with severe acute respiratory syndrome (SARS).

Both viruses belong to the Coronaviridae family. They are characterized by a single-stranded 30 kb positive-sense RNA and enveloped spherical virions of about 160 nm. The unusual large size of their genome leaves these viruses enough space to rearrange their genes (recombination), thus donating them some genomic plasticity [5]. Furthermore, RNA biosynthesis seems to use a virus-specific template switch, which results in transcription of sub-genomic mRNAs and eventually leading to homologous RNA recombination [5]. Nevertheless, by encoding a 3'-5' exonuclease within non-structural protein 14 (nsp14-ExoN), which is required for high-fidelity replication, the mutation capacity of

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SARS-CoV-2 is debated [6]. In the end, somehow the plasticity allowed Coronaviridae to acquire a rich strains biodiversity and the ability to jump species, which had already caused previous zoonotic outbreaks, such as for MERS-CoV and SARS-CoV [7–9].

Starting from observed similarities in a short region of *RdRp* gene between SARS-CoV-2 and a bat coronavirus (BatCoVraTG13), further sequences were identified to be 96% identical at the whole-genome level, corroborating the hypothesis of animals to humans spillover [10].

As of 3 June, more than 6 million cases of COVID-19 have been declared, including more than 380 000 deaths [11]. Because of the rapid and fatal spread of the pandemic, the research on development of diagnostic tests was set as a priority for infection control measures and patient care.

The present review summarizes performances and limitations of diagnostic tests to help clinicians in the interpretation of the results and clinical management.

Diagnostic tests for SARS-CoV-2

Nucleic acid amplification tests (NAATs)

Three of the major challenges in molecular diagnosis are (a) to detect small amounts of viral RNA for reducing the number of false negatives, (b) to differentiate the positive signal among different pathogens for decreasing the number of false positives and (c) to have a large capacity, in order to quickly and correctly test a large number of patients, while avoiding false negatives and false positives. Molecular and serological tests were previously compared during the SARS-CoV-1 epidemic, showing an increased sensitivity and specificity for the molecular ones. For this reason, real-time reverse transcription polymerase chain reaction (rRT-PCR) represents the validated assay for early diagnosis in patients with suspected SARS-CoV-2 infection [12].

First publications showed that diagnosis was possible by targeting the spike (*S*) gene of the virus with a good specificity (differentiating SARS-CoV-2 from SARS-Cov-1), but limited sensitivity [10]. Sensitivity was further improved when integrating other viral-specific genes, such as *RdRp*/Helicase (*Hel*), Nucleocapsid (*N*) and Envelop (*E*) genes [13]. A comparison between all targeted genes revealed that the best results were obtained with *RdRp*/*Hel* genes [14], and WHO guidelines recommend the use of *RdRp*, *E*, *N* and *S* genes in different combinations [12]. In our institution, we introduced the WHO recommended test described by Corman et al. (targeting the *E* gene, followed by confirmation with *RdRp* primers) [13] on our fully automated molecular diagnostic platform [15]. RNA extraction was performed through the MagNA Pure 96 System (Roche, Basel, Switzerland) and the rRT-PCR was carried out on a QuantStudio 7 system (Applied Biosystems, Waltham, USA) [15]; we observed low sensitivity of the rRT-PCR targeting the *RdRp* gene due to a mismatch [16] in the primer described by Corman et al. [13] and, since the *E* gene was constantly more sensitive than *RdRp*, we decided, after having performed about 1000 tests, to continue addressing the *E* gene only [16]. This allowed us to save reagents and to be able to perform an increasing number of tests in a setting of reagent shortage due to the pandemic nature of Covid-19. To detect a possible drift due to mutations and to avoid that a mutant strain escape our diagnostic test, we kept targeting both *E* and *RdRp* genes once a week.

RNA extraction methods can generally be classified into (a) one step (with the RT step and the PCR reaction in the same tube) and (b) two-step RT-PCR (initial creation of DNA copies with RT reaction followed by their addition to the PCR reaction). Typically, one-step PCR uses one reaction tube, minimizing the risk of contamination

(false-positive results). On the other hand, two-step PCR allows the cDNA sample to be archived and further testing of other genes.

As pointed out in a recent work from Pang et al. [17], there are different RNA extraction platforms (summarized in Table 1), which can allow sensitivity and TAT to be improved but also sometimes increasing the costs.

Despite the good performance of the validated nucleic acid amplification tests (NAATs), there is still a risk of false-negative results. Most of them concern the pre-analytic setting, such as the timing of the specimen collection (too early or too late in the infection course, including the limit of detection due to late infections with atypical manifestations), the quality of sampling (insufficient material) or type of specimens (bronchoalveolar lavage (BAL) exhibits the highest sensitivity, followed by induced sputum, nasopharyngeal (NP) swab, oropharyngeal (OP) swab and faeces), and finally the sample transport (inappropriate container, exposure to extreme temperatures, etc.) [18,19]. Further reasons decreasing the performance of a molecular test include the ability of the virus to mutate (changing sequence of the regions in which primers are hybridizing), or the PCR inhibition [20], the latter being nowadays almost insignificant (in our laboratory, we observed an inhibition in about 0.3% of the tested samples).

With the purpose of reducing the TAT of NAATs, several rapid microarray and sequencing solutions built on multi-RT-PCR panels (and automated systems) have been developed [17,21]. An important highly performing test, although expensive and not useful for a high number of samples, is the GenXprt SARS-CoV-2 test (Cepheid, <https://www.cephheid.com>), which due to its simplicity and its very short TAT, rapidly helped patient management especially regarding isolation procedures [22].

Finally, several manufacturers already developed other advanced specific systems [23–28] and investigations showed their easiness, rapidity and compatibility with automation [29,30].

Serological testing

One of the downsides of serological assays is the limited sensitivity at an early stage, when the host has not yet developed specific antibodies. In the specific case of SARS-CoV-2, data from literature showed production of IgM and IgG starting after the first week from infection and generally detectable from the second [31–34], leaving some space for delayed antibody responses, previously associated (for MERS-CoV) with more severe disease [35].

Another conundrum is to avoid the cross-reactivity between antibodies against different viruses, part of the same or different families. This was a concern since most of the human coronaviruses are antigenically closely related [36], although solvable by adding a virus neutralization test (as previously suggested by WHO during the SARS epidemic in 2004) [37]. However, most current serology tests exhibit a specificity greater than 98%.

Sensitivity and specificity of serological assays can also be affected by the target antigen. As highlighted by Meyer et al., the *S* protein (produced in more advanced stage of SARS-CoV-2 infection) showed lower levels of sensitivity and more specificity (especially the S1 subunit) than the *N* protein [38]. We recently observed (data not shown) that the antibodies directed against the *N* protein seems to decrease earlier than the *S* protein; thus the sensitivity of assays targeting only the *N* protein may be impaired according to the timing of infection (Fig. 1). For this reason, we recommend to systematically use two tests, one targeting the *S* protein and one targeting the *N* protein for diagnostic purposes. For sero-epidmiological studies, a test targeting the *S* protein is recommended. The added value to target the *S* protein is that the titres are likely to better reflect protection against reinfection. In Table 2 we summarize the interpretation of diagnostic microbiological

Table 1
Advantages and disadvantages of molecular diagnostic methods for detection of SARS-CoV-2

NAAT extraction method	Advantages	Disadvantages
rRT-PCR	Reference method, high sensitivity and specificity, compatibility with automation and multipanels	Long TAT without automation
Nested PCR	Increased sensitivity due to the added pre-amplification step	Longer TAT and lower specificity due to the higher risk of contamination
RT-LAMP	Shorter TAT	Possible slightly lower sensitivity
RT-iiPCR		Possible slightly lower sensitivity
Gene expert	Automation, high sensitivity and specificity, molecular rapid test	High costs, limited number of samples per time
MNPs-based methods	Increased rapidity, compatibility with automation	

rRT-PCR, real-time reverse transcription polymerase chain reaction; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-iiPCR, reverse transcription insulated isothermal polymerase chain reaction; MNP, magnetic nanoparticle; TAT, turn-around-time.

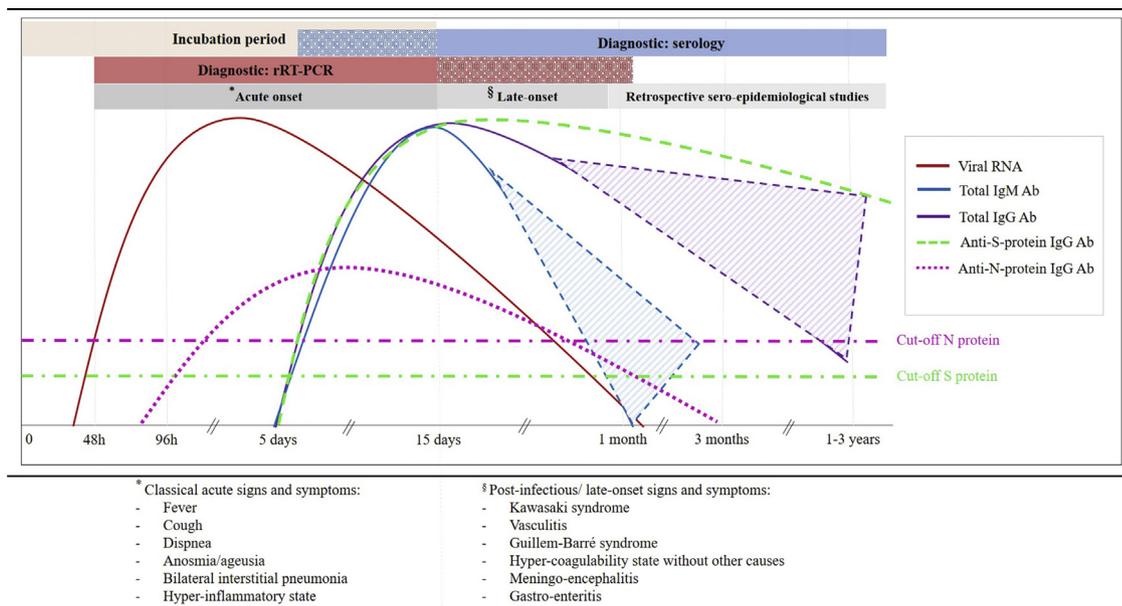


Fig. 1. Kinetics of SARS-CoV-2 markers during infection and laboratory diagnosis. rRT-PCR, real-time reverse transcription polymerase chain reaction; RNA, ribonucleic acid; IgM, immunoglobulin type M; IgG, immunoglobulin type G; Ab, antibodies.

tests according to the time of infection, the presence or absence of symptoms and the type of diagnostic test used. Interestingly, the high specificity of the S protein was corroborated by a study on SARS-CoV-2 spike protein epitopes showing that, even considering the homologies with SARS-CoV-1, the virus of COVID-19 exhibits novel antibody epitopes [39]. This translates in the lack of efficacy of SARS-CoV-1 antibodies against COVID-19 [40], but at the same time it reinforces our trust in the specificity of the serological test when targeting S proteins.

There are currently a huge number of tests on the market [21]. Ideally, only high-quality assays with sensitivity greater than 95% and specificity superior or equal to 98% should be used. As well as for NAATs, different platforms can be considered also for serological tests. The lateral flow assays (LFAs) often exhibits a lower sensitivity than the enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immune-assays (CLIAs) [41]. Among the different LFAs, we identified the Dynamiker (Tianjin) as one of the best, being both sensitive and specific (Coste et al., 2020, data not shown, submitted). Nevertheless, we preferred ELISA and CLIA, which enable larger series and less hands on time. In Lausanne, we initially started on 14 April using a ELISA-based assay (Epitope Diagnostics, USA) that was targeting the N protein; this ELISA exhibited 96% of sensitivity on samples taken 15–30 days post

infection and a specificity of 100% on 450 samples taken before the outbreak (Coste et al., 2020, data not shown, submitted). More recently, we moved to CLIA. The Snibe run on Maglumi instruments (China) target the N protein, like the Roche CLIA, running on Cobas instruments (Switzerland). Conversely, the Abbott test running on the m2000 instruments (USA) and the Diasorin test running on the Liaison instruments (Italy) both target the S protein.

As mentioned, median time for seroconversion is about 10–14 days [31–33], but early seroconversion has also been documented at 3–5 days post infection [34]. Interestingly, the appearance of IgM occurs at the same time as IgG but IgMs last for a

Table 2
Clinical interpretation of microbiological diagnostic results

Symptoms	rRT-PCR	IgM	IgG anti-S	IgG anti-N	Interpretation
+/-	+	-/+	-/+	+/-	Acute infection
+	-	+	+	+	Recent infection
+	-	+	+	+	Late onset infection
-	-	-	+	+/-	Old infection
-	-	-	-	-	Absence of infection

rRT-PCR, real-time reverse transcription polymerase chain reaction. +/-: often positive. -/+: possible to be positive.

Table 3
Summary of indications for serological testing

Indication and usage	Explanation
Help define the prognosis of a given subject Solve discrepancies between clinical presentation and RT-PCR results	Low IgG/IgM levels at 15 days post-infection might correlate with immunodeficit Despite high pre-test probability due to suspicious clinical presentation, molecular diagnosis might still results negative in: <ul style="list-style-type: none"> • Covid-19 complications (i.e. Kawasaki or Guillain–Barré syndrome, vasculitis, thrombo-embolic event) • Late-onset disease (i.e. meningo-encephalitis or gastroenteritis)
Solve discrepancies between different molecular tests	Different tests might be used, together with clinical presentation, as external gold standard in order to build the positive group upon validation of a new molecular test
Retrospective diagnosis of SARS-CoV-2 infection	SARS-CoV-2 infection potentially correlates with (transient?) acquired immunity
Sero-epidemiological studies	To help assessing the magnitude of an ongoing outbreak (pandemic) and its spread rate
Support therapy perspectives	Identify individuals with very high antibody titres that may be selected for blood donation to derive large amount of immunoglobulins for passive immunotherapy treatment

shorter time; thus, the main advantage to also test IgM is to assess the timing of the infection.

The need of developing point-of-care devices to reduce TAT and increase the number of daily tests have stimulated the research towards faster and simpler kits. Numerous manufacturers quickly developed rapid immunochromatographic point-of-care tests, already available on the market [21].

At this point, data are being gathered on the diagnostic gain obtained through these rapid kits, and further and more accurate studies are warranted, which can define sensitivity and specificity of the tests in relation with timing of infection and targeted proteins.

Discussion

In a complex scenario such as the ongoing pandemic, not only the diagnoses need to be timely and accurate, but laboratory testing needs also to provide epidemiological information, in order to assess the magnitude of the event and the spread rate. In this setting, serological assays can become helpful both to complete the epidemiological link when molecular diagnosis results are negative, and to alleviate the burden of laboratories implicated in molecular diagnosis [12]. In this way, a rapid point-of-care serological assay, cheap and simple enough to be affordable by any hospital, if used correctly (not at an early stage of disease), could allow enlarging the spectrum of the tested population, especially among those who did not develop enough symptoms to require hospitalization, hence molecular testing. At the same time, if used as an asset for population screening at the right time and as adjunctive to the reference method, the overall epidemiological information gained could account for the sensitivity loss in early stages of diagnosis. Indications for serological testing are summarized in Table 3.

While serological assays can represent a useful epidemiological asset, NAATs remains the reference standard for diagnosis because of their high sensitivity even at early stages of the disease.

Following the quantification of viral load over time and integrating that with information on sample collection technique and timing might be helpful to differentiate different stages of the disease. Viral quantification will also provide information regarding the value of testing different samples from different parts of the body.

Owing to the main respiratory tropism of SARS-CoV-2, the best samples (for sensitivity) come from the respiratory tract: in particular, NP swabs showed higher and longer persisting viral loads than OP swabs [42].

Because of the higher concentration of the virus in the lower respiratory tract, it could be argued that the sensitivity of NP swabs

is still not enough to avoid false-negative results. Samples from lower respiratory tract, such as BAL or bronchial aspirates require invasive procedures, not possible without intubation in subjects already suffering from severe respiratory insufficiency.

Finally, the decision whether to trust or not a negative NP swab should be always corroborated by the clinical presentation of the patient [43] and, if possible, completed by serology testing. If a strong clinical suspicion is present, repetition of the test is recommended. A new NP swab is the first option, since often the false-negative NP test is due to inadequate sampling of only the distal part of the nasal cavity. Repetition of the test with a lower respiratory tract sample will be advisable for those patients admitted to the hospital, hence with heavier symptoms, but stable enough to be submitted to invasive procedures. If the patient is asymptomatic but still considered at risk of infection due to specific exposure, investigating the serology between 10 and 15 days after the exposure could help to document an asymptomatic infection.

Samples like blood and urine were found to be weakly to not sensitive, while the virus was also found in faeces and perineal swabs of patients with gastro-intestinal symptoms [44,45], indicating another sample deserving further investigation.

Another consideration is whether the local (respiratory, gastro-intestinal) microbiota play a role against the diffusion of the virus, which, together with the immune system diversity, might contribute to the variety of clinical presentations and affect different viral concentrations [46,47].

Finally, considering this rapidly evolving pandemic and its zoonotic outbreak, more knowledge needs to be gathered from further studies in the diagnostic field, both investigating the specific features of this virus and novel diagnostic approaches, possibly combining the molecular methods with the serologic ones in order to booster sensitivity and specificity results. Despite the desperate need to implement NAAT or serological test, the introduction of these tests needs to be done with high quality criteria to assess the performance of the tests in order to avoid a massive amount of false positive and false negative results. To save time, the validation of these tests should be centralized to reference laboratories and then rapidly implemented in other laboratories upon validation of the performance of the test.

Transparency declaration

Dr Caruana, Dr Croxatto, Dr Opota, Dr Coste, Dr Lamoth, Dr Jatón have nothing to disclose. Dr Lamoth reports personal fees from Gilead (U.S.A), personal fees from MSD (U.S.A), outside the submitted work. Dr Greub reports grants from Resistell (Switzerland), from Nittobo (Japan), outside the submitted work. The authors did not receive any financial support for this work.

Author contributions

Dr Caruana conceived and design the study, performed the literature research, drafted and revised the article, and finally approved the version to be submitted. Dr Croxatto, Dr Oputa, Dr Coste, Dr Lamoth, Dr Jatón contributed to the conception and design of the study, critically revised the article and finally approved the version to be submitted. Prof. Greub conceived and designed the study, contributed to drafting the article, critically revised the article and finally approved the version to be submitted.

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