

BOLETIM RAMB COVID-19

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Uses and limits of the clinical laboratory in the COVID-19 pandemic: a didactic review

Fernando Antonio Glasner da Rocha Araujo

Em um momento em que há uma emergência mundial de saúde pública, é fundamental que o conhecimento científico gerado durante a pandemia chegue rapidamente à classe médica classe médica.

Dentro desta dinâmica a Revista da Associação Médica Brasileira (Ramb) está adotando uma série de medidas a fim de acelerar o processo editorial para publicação de artigos sobre a Covid-19. A partir de hoje (14/04/2020), a AMB publicará o Boletim Ramb Covid-19, que antecipará os artigos científicos selecionados pelos editores da Ramb sobre o tema.

“Os artigos foram escritos por especialistas e selecionados dentro dos critérios da Ramb para esclarecer temas fisiopatológicos, assim como oferecer orientações de prevenção e tratamento da doença. Dessa forma, esperamos colaborar com os médicos para o melhor atendimento aos seus pacientes, com a disponibilidade mais ágil desses artigos, antes de sua publicação na Ramb”, comenta Carlos Serrano Jr., editor-chefe da Ramb.

Para o diretor científico da AMB, Antonio Carlos Palandri Chagas, “neste momento ímpar vivido no mundo por conta da pandemia de Covid-19, a AMB cumpre seu papel de estar levando à comunidade científica brasileira os recentes artigos sobre os mecanismos fisiopatológicos e aspectos clínicos relevantes dessa situação que assola a saúde pública”.



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Uses and limits of the clinical laboratory in the COVID-19 pandemic: a didactic review

 Fernando Antonio Glasner da Rocha Araujo¹

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SUMMARY

The world is currently experiencing an unprecedented pandemic of a new disease, the coronavirus disease (COVID-19), which has unusual clinical and immunological presentations. This is especially true regarding the choice and interpretation of laboratory test results. In this review, we have provided didactic information for physicians on the current concepts and practical guidance regarding COVID-19.

KEYWORDS: Severe acute respiratory syndrome - coronavirus 2, COVID-19, clinical pathology, clinical diagnosis, laboratory.

RESUMO

PALAVRAS-CHAVE: síndrome respiratória aguda grave - coronavírus 2, COVID-19, patologia clínica, diagnóstico clínico, laboratório.

INTRODUCTION

The Coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 was first identified after an outbreak of pneumonia of unknown etiology in Wuhan, Hubei Province, China in December 2019.¹

Laboratory tests can play different and important roles in medical decision making during the current COVID-19 pandemic by providing the following:

- Etiological diagnosis of the disease
- Serological diagnosis of the disease
- Immunological status evaluation
- Severity and/or prognosis indicators

The choice of test depends on the inherent characteristics of the test (sensitivity, specificity, and predictive values), the characteristics of the target population (prevalence, incidence, and pre- and post-test probability), and a combination of both (likelihood ratio).

Since COVID-19 is a novel disease, the current knowledge regarding its characteristics, especially laboratory characteristics, is limited. In this review, we have provided didactic information on the current concepts and practical guidance regarding COVID-19 based on the current knowledge to help physicians choose laboratory tests and interpret their results based on different clinical presentations.

METHODS

A literature review was performed to identify studies that met the objective of the research. The following strategies were used. First, a literature search was performed in PubMed and SciELO using the terms SARS-CoV-2, COVID-19, laboratory, and interpretation. Second, an active Google database search was performed using the specific terms SARS-CoV-2, COVID-19, laboratory, interpretation, polymerase chain reaction, and serology. Third, websites of several national (such as Ministry of Health, Brasil

and Scientific Societies of Clinical Pathology and Infectious Diseases) and international (such as CDC-USA) scientific and government entities were searched for recommendations or practice guidelines. Lastly, articles cited in the previously selected articles and strategies were explored. We searched for articles published in 2020 in English, Portuguese, or Spanish.

RESULTS AND DISCUSSION

Using the aforementioned search strategy, 107 articles were initially found in the databases and 6 on the websites. After excluding the articles that did not meet the study criteria and including 4 articles referenced in previously selected articles, 24 articles were finally analyzed (Figure 1).

FIGURE 1

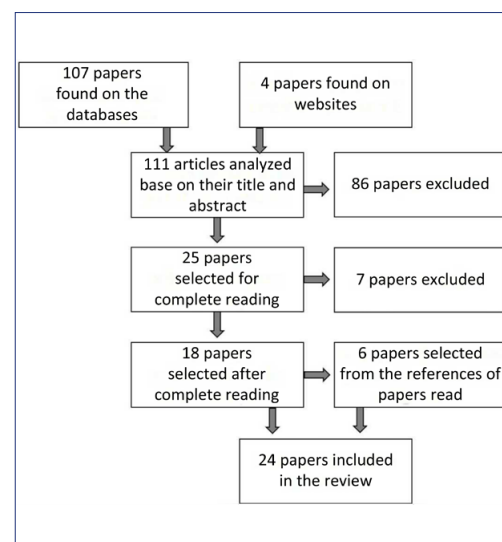
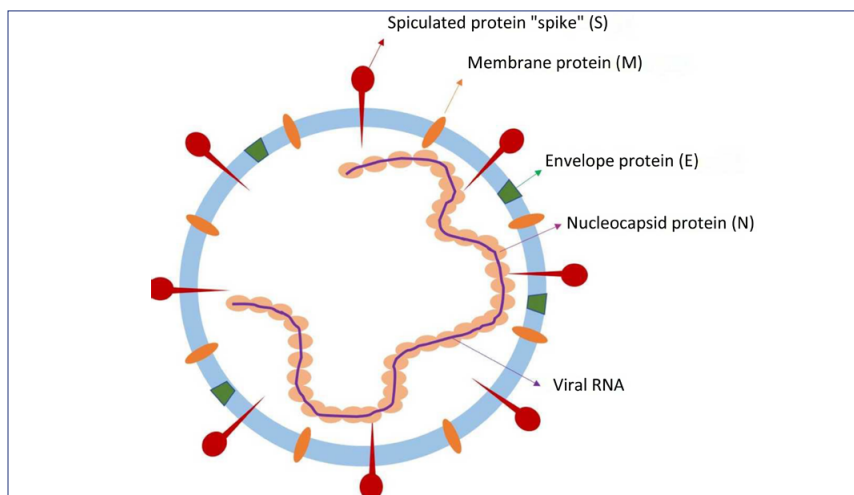


FIGURE 2



Etiological diagnosis (molecular methods)

The structure of SARS-CoV-2 (Figure 2) consists of a single RNA ribbon in the center surrounded by a nucleocapsid (N). This structure is wrapped in a lipid-membranous layer (M) that has different proteins, such as envelope (E) and spiculated or spike (S) proteins, which gives the virus the appearance of thorns or tips of a crown (origin of the term *corona*).² Molecular tests are based on the identification of the genes that encode these proteins using reverse transcription-polymerase chain reaction (RT-PCR). Currently, the genes used for identification are those coding for E, N, S, and RNA-dependent RNA polymerase. The tests usually identify more than one genetic marker, and most of them search for genes coding for N and E.

Reverse transcriptase-polymerase chain reaction

RT-PCR is the most widely used method for genetic identification of the virus and is considered the gold standard.

The ideal use of this test depends on factors such as the time and site of sample collection.

Kucirka et al.³ reported that the best time to collect samples for SARS-CoV-2 RT-PCR is between the third and fourth days after symptom onset

(Table 1). Further, they reported that all patients tested in their pre-symptomatic phase were negative; therefore, testing asymptomatic patients would not be justified. However, Sakurai et al.⁴ observed that during the COVID-19 outbreak on the Diamond Princess ship, 712 of the 3,711 people aboard tested positive for COVID-19. Moreover, 410 of 712 (58%) people were asymptomatic at the time of sample collection. These two reports, although containing contradictory findings, represent two distinct pre-test conditions. The first includes patients who, with no history of suspicious contact, wish to undergo the RT-PCR test to know if they were infected. The second includes an important precedent of prior contact. Therefore, only the latter scenario would justify the testing of asymptomatic individuals, considering that about half of them would test negative. The duration from the initial test to symptom onset was a mean of 4 days (range, 3–7 days).⁴

Furthermore, only 8 of 32 (25%) people on the Diamond Press ship who were cabin companions of COVID-19-positive patients subsequently tested positive.⁴ This justifies the frequent observation of different results among family members or people sharing the same household with COVID-19-infected persons.

Almost all published studies are

based on the collection of nasopharyngeal samples, wherein the sensitivity varies between 78% (for 1 test) and 86% (for 2 tests), with a specificity of about 99%. Some authors have highlighted the increase in sensitivity for COVID-19 diagnosis when RT-PCR is used in combination with chest computed tomography (sensitivity of 91.9%).⁵

A positive RT-PCR test probably means the actual presence of viral infection (true positivity), since false-positive results are very rare. False-positive results are most likely due to a processing error with the contamination of the tested sample. Test results that are not false-positive effectively identify viral genetic materials; however, it is necessary to consider the possibility of the occurrence of these materials even in the absence of viral replication, since the virus could be in an inactive state.⁶

The main limitation of this examination is the significant number of false-negative results because of diverse reasons such as:

- The viral load in secretions and excretions depends on the stage of infection; it is lower in samples collected less than 3 and more than 10 days after the onset of infection (Table 1).³
- The classical collection sites (nose and oropharynx) tend to show less positive tests than those collected in the lower respiratory tract (such as bronchoalveolar lavage); however, the collection technique

TABLE 1. PREVALENCE OF FALSE-NEGATIVE RT-PCR TEST RESULTS FOR SARS-COV-2 BASED ON THE TIME OF SYMPTOM ONSET

Time	False-negative results
4 days prior to symptom onset	100%
1 day after symptom onset	67%
3 days after symptom onset	20%
4 days after symptom onset	21%
16 days after symptom onset	66%

Kucirka et al., 2020³

of the latter is more complex and not available in most laboratories (Table 2).⁷

- Degradation of the sample during transportation and storage before analysis.

Many studies have reported the detectable presence of viral particles in the saliva, blood, feces, and urine; however, there are no routine protocols for diagnosis using these viral particles.⁸ Viral particle detection in the urine is very rare.⁹

A possible explanation for the difference in positivity between these different samples is the time taken to express viral replication. Most of the current knowledge comes from RT-PCR test results from the analysis of samples collected from the nasopharynx. Wölfel et al.¹⁰ described that sputum samples show positivity even after the virus is no longer detected in nasopharynx samples on RT-PCR. Zhang et al.⁹ demonstrated that the RT-PCR test results for fecal samples remain positive for a longer

period than those for nasopharyngeal samples.

The reason why viral material persists for more than 14 days in some samples and its relationship with the possibility of disease transmission is unclear.

Generally, since the occurrence of false-positive results is very rare, a positive RT-PCR test result can be considered a case of SARS-CoV-2 contamination. However, when analyzing a strongly suspected COVID-19 case (high pre-test probability) that presents a negative RT-PCR test result, it is recommended to repeat the test at least once, and if possible, it is recommended to collect the sample from the lower respiratory tract.¹¹ Even if the results are negative, considering the high pre-test probability, it is better to isolate the patient.¹¹

Watson et al.¹¹ calculated pre-test and post-test probabilities using the sensitivity (70%) and specificity (95%) data for RT-PCR in the literature (Table 3).

Reverse transcriptase loop-mediated isothermal amplification polymerase chain reaction

Although less common than RT-PCR, the RT loop-mediated isothermal amplification PCR (LAMP) technique has some advantages such as faster execution time, simpler reading (visual), the possibility of measurement at the point of care, and the possibility of running more tests simultaneously.¹² Moreover, it presents a high specificity for SARS-CoV-2, and no cross-reaction with other coronaviruses (such as HCoV-229E, HCoV-NL63, HCoV-OC43, and MERS-CoV), influenza viruses (such as type B, H1N1pdm, H3N2, H5N1, H5N6, H5N8, and H7N9), or other respiratory viruses (such as RSVA, RSVB, ADV, PIV, MPV, and HRV).¹³

However, this technique needs higher viral loads, which reduces its detection limit, and is qualitative.⁶

At first, this would be the technique of choice for population use.

Serological diagnosis

Serological tests are based on the detection of antibodies produced against viral antigens. These tests can detect total or specific antibodies (IgM, IgG, and less commonly, IgA).

Antibody detection depends on the time elapsed since infection onset. IgA and IgM antibodies are usually detectable in the first 7 to 10 days of infection, whereas IgG can be detectable after about 10 to 15 days of infection. Ideally, these antibodies peak after the third or fourth week of illness.¹⁴

Patients whose clinical presentations are compatible with suspected COVID-19 and those with RT-PCR-confirmed COVID-19 present a positive antibody testing rate after 14 days of disease onset ranging from 50% to 100% (mean 72%) for IgM and 64.7% to 100% (mean 91%) for IgG. In other words, even in patients positive for COVID-19 on RT-PCR, about 10% do not present positive IgG; they constitute the so-called “false negatives” (Table 4).¹⁵

The reason why these patients present no seroconversion is unknown. These patients seem to show a similar tendency to that of those who develop no anti-HB antibodies even after

TABLE 2. SENSITIVITY BY COLLECTION SITE IN SARS-COV-2 CARRIERS

Collection site	Sensitivity
Bronchoalveolar lavage	93%
Sputum	72%
Nasopharynx	63%
Oropharynx	32%

Wang et al., 2020⁷

TABLE 3. POST-TEST PROBABILITY OF POSITIVE AND NEGATIVE RT-PCR RESULTS

Pre-test probability	Post-test probability of 1 negative result	Post-test probability of 2 negative results	Post-test probability of 1 positive result
5%	1.6%	0.5%	42%
15%	5%	2%	71%
25%	10%	3%	82%
50%	24%	9%	93%
75%	49%	23%	98%
90%	74%	47%	99%

Watson et al., 2020¹¹

TABLE 4. PERCENTAGE OF IGG SEROCONVERSION BASED ON THE TIME FROM INFECTION

1 st author	Technique	IgG		
		Yes	No	%
Gao	CLIA, ELISA, GICA	14	0	100%
Jiang	Proteome microarray	29	0	100%
Yong	GICA	35	3	92%
Liu	In-house kit	131	2	98%
Long	MCLIA	285	0	100%
Lou	ELISA, LFIA, CMIA	75	5	94%
Pan	ICG strip	65	2	97%
To	EIA	16	0	100%
Zhao	ELISA	112	61	65%
TOTAL	TOTAL	762	73	91%

Flodgren et al., 2020¹⁵

repeated immunization attempts with a hepatitis B vaccine.

The percentage of positive antibody tests does not seem to depend on clinical severity.¹⁵

In addition to false-negative results, false-positive results can occur by cross-reaction with other viruses. This is more common when IgM or IgG titers are very close to the cutoff point. In the case of false-positive results, it is recommended to repeat serum tests after 2 weeks. In the case of a true-positive reaction, a significant increase in IgG titers (double or more) is expected. In false-positive cases, IgG tends to be negative in the second sample analysis.

Remote Laboratory Tests (rapid tests)

Remote laboratory tests (RLTs) or point-of-care tests, also known as rapid tests, are performed outside the laboratory setting. They are aimed at rapidly screening for the presence of antibodies and do not require the expertise of trained personnel. Generally, they are based on immunochromatography techniques using whole blood on substrates assembled in molded plastic (soap type).

Their simple interpretation, easy technique, and rapid performance make them, at least theoretically, a very useful diagnostic tool (Figure 3). However, the performance characteristics reported by the manufacturers during validation are not standardized. Moreover, they do not provide information on the characteristics of the population tested. Many samples have presented inappropriately low-reliability data, resulting in tests with low accuracy and making their usefulness in clinical practice unfeasible.¹⁶

For these reasons, the World Health Organization recommends the use of RLTs for research purposes only, including public health surveys. Rapid tests are used to estimate disease seroprevalence in a given population.¹⁷

Automated laboratory tests

Automated laboratory tests are performed inside the laboratory using automated analytical equipment by trained personnel and ideally under the supervision of an experienced clinical pathologist. These tests are usually quantitative and have higher quality (accuracy, reproducibility, sensitivity, and specificity) than rapid tests.

Several methodologies have been used, but the most common are enzyme-linked immunosorbent assay (ELISA), chemiluminescence (CLIA), and electrochemiluminescence (ECL).

A comparison between these techniques shows that ECL is faster to implement, more sensitive, and more specific than the other automated techniques.¹⁸ However, the technique identifies total antibodies, without distinguishing between IgM or IgG classes.

ELISA and CLIA, although less sensitive, distinguish between antibody classes. Therefore, it would be ideal to initially use ECL, and in positive cases, perform the ELISA or CLIA.

Immunological status evaluation

Typically, the presence of antibodies is interpreted as follows. IgM: These are usually interpreted as indicators of the initial phase of the immune response and, therefore, of recent infection. However, with the introduction of increasingly sensitive tests, it has become common to detect IgM antibodies weeks and even months after

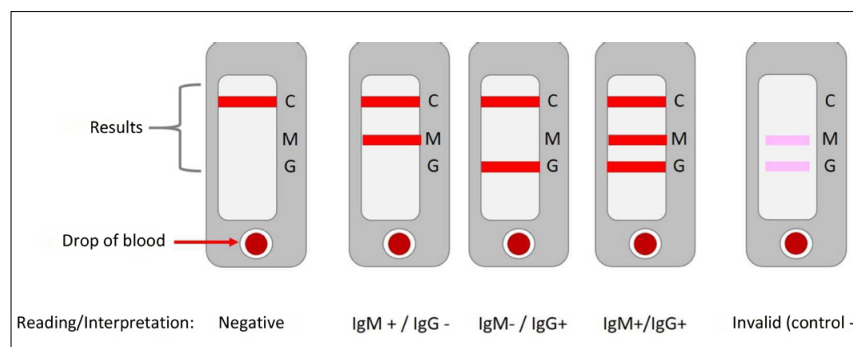
infection. It is also necessary to consider that antibodies of this class are less specific than IgG. Therefore, IgM positivity may be related to a cross-reaction with antigens of other viruses. IgG: These are usually interpreted as indicators of patient immunization; however, with COVID-19, this interpretation has been questioned. In addition to the large number of patients who are not IgG positive, it has been described that infected asymptomatic patients produce less-lasting antibodies.¹⁹ The implications of these findings regarding the nature or duration of immunity and the efficacy of response to new viral attacks in the future is unclear.

A possible explanation for differences between IgG positivity and immune status, in Covid-19, is the type of target antigenic determinant the antibodies produced. Sethuraman et al.¹⁴ stated that most antibodies produced (and detected in assays) are directed toward the most abundant viral protein—the N protein. Thus, tests detecting the N protein are more sensitive; nonetheless, these are possibly not neutralizing antibodies and they may not indicate immunity. Conversely, antibodies directed toward the receptor-binding domain of the S protein are more specific and possibly neutralizing.

Atypical behaviors of anti-SARS-CoV-2 antibodies have been reported by Brazilian clinical pathologists²⁰:

- IgM antibodies tarry for more than 7 weeks, with no set time for negativity

FIGURE 3



- False-negative or indeterminate IgG results up to 50 days after the symptom onset with RT-PCR positivity
- IgG results that become positive 20 days after symptom onset, with slow growth and no prediction of reaching the IgG concentration plateau
- Some patients present no IgM positivity even in the active phase of infection

In addition to the clinical diagnosis of recent infection (IgM positivity) or immunization (IgG positivity) commonly used by doctors to follow-up cases, the Brazilian Society of Clinical Pathology/Laboratory Medicine²¹ highlights the following situations wherein these tests may be useful:

- The diagnosis of hospitalized patients with late clinical presentation (after the seventh day of symptom onset) as the first option before the PCR reaction. However, a negative result in this context does not rule out the diagnosis of COVID-19 and specific molecular testing (RT-PCR) is recommended.
- Return to work evaluation for health professionals from the seventh day of symptom onset. As previously stated, a negative result does not exclude the diagnosis of COVID-19 and RT-PCR is recommended.

Severity and/or prognosis indicators Ruan et al. described mortality predictors in 150 patients with COVID-19. Some of the clinical predictors were mean age (67 vs. 50 years), presence of comorbidities (especially cardiovascular disease, renal failure, respiratory failure, and associated infections), and disease severity (need for ICU admission and life support). The laboratory parameters associated with a fatal outcome described were leukocytosis (10,620 vs. 6,760/mm³), lymphopenia (600 vs. 1,420/mm³), thrombopenia (173,600 vs. 222,100/mm³), evidence of renal failure (increased blood urea nitrogen

and serum creatinine levels), changes in muscle enzyme levels (myoglobin and cardiac troponin levels), and changes in inflammatory response markers (decreased albumin levels and increased C-reactive protein, ferritin, and IL-6 levels).²²

Coagulopathy is a recognized risk factor for COVID-19 mortality and expressed by significantly increased levels of D-dimer and fibrin degradation products.²³

CONCLUSION

Table 5 shows the different clinical and epidemiological situations that physicians might encounter while treating patients with COVID-19 and the procedures that are most supported by current knowledge.

The beginning of the interpretation is based on the elapsed time since symptom onset.

In the absence of symptoms, the interpretation is based on the time elapsed since close contact with a

SARS-CoV-2 carrier, evidenced by molecular testing. The more intimate and prolonged the contact, the more significant the history. However, the concept of close contact with a SARS-CoV-2 carrier can be quite broad, and includes the following²⁴:

- A person who had direct physical contact (e.g., shaking hands).
- A person who had unprotected direct contact with infectious secretions (e.g., cough droplets, unprotected contact with used tissue or tissues containing secretions).
- A person who had face-to-face contact for at least 15 min and at a distance of at least 2 m apart.
- A person who was in an enclosed environment (e.g., classroom, meeting room, hospital waiting room, etc.) for at least 15 min and at a distance of at least 2 m apart.
- A health care professional or another person directly handling a COVID-19 case or laboratory workers handling samples from

TABLE 5. CHOICE OF TESTS IN DIFFERENT CLINICAL EPIDEMIOLOGICAL SITUATIONS

Patient clinical and epidemiological	Reason for test	First-choice test	What to do if the result is negative
Asymptomatic with no history of contact with carriers	Find out if the patient has been contaminated before	No indication for testing	-
	Assess the population incidence of virus infection/immunization	RLT "Rapid Test" (immunochromatography)	-
Asymptomatic after contact with COVID-19 patient	Define quarantine requirement	RT-PCR	Serology after 15-30 days of contact
Patient with early symptoms suggestive of COVID-19 (up to 7 days from symptom onset, ideally between 3-4 days)	Diagnosis of the disease	RT-PCR	Serology (after 15 days of symptom onset)
Patient with symptoms suggestive of COVID-19 with late symptom onset (after day 7 and before 14 days of symptoms)	Diagnosis of the disease	RT-PCR + chest CT	Serology (after 15 days of symptom onset)
Symptomatic or recovered patient, more than 15 days after symptom onset		Serology	
The patient recovered from COVID-19, after 7 days	Evaluate return to work of health personnel	RT-PCR	Serology
Patient recovered from COVID-19, more than 14 days after the first symptom onset	Diagnosis (retrospective) of the disease	Serology	

a COVID-19 case without recommended personal protective equipment (PPE) or with a possible PPE violation.

- An aircraft passenger seated within a radius of two seats (in either direction) from a confirmed case of COVID-19, his companions, or caregivers, and the crew members who worked in the aircraft section where the patient was seated.

- A person who lives in the same house/environment. Residents of the same house, dormitory, nursery, and accommodation should be considered.

Finally, we hope that this text will be useful and contribute to the better use of the laboratory in the diagnosis of SARS-Cov-2. Much of the knowledge about this condition will continue to evolve in the coming months,

adding or changing part of what we have reviewed today. ■

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