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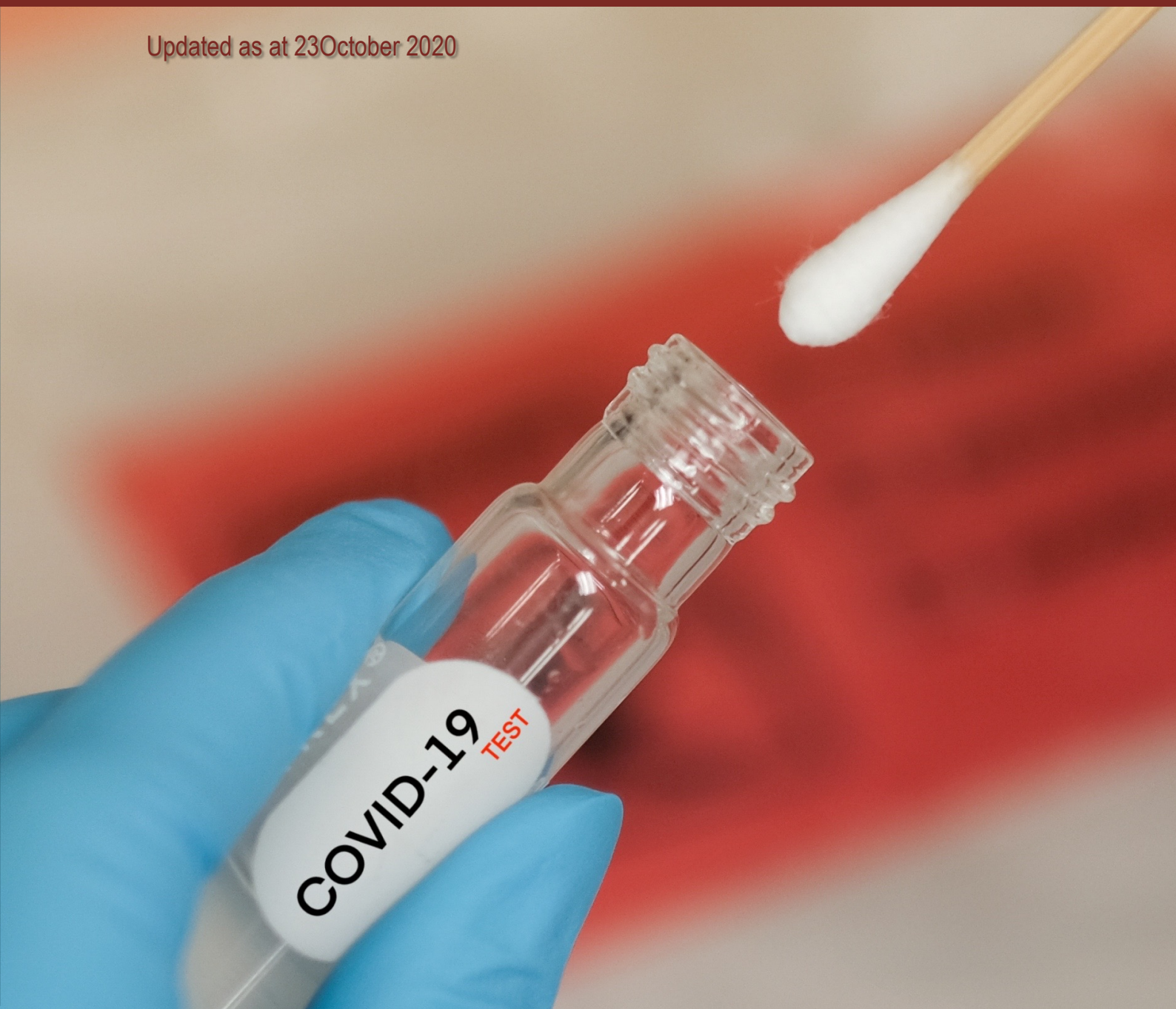
INTERIM TECHNICAL NOTE

Laboratory tests for SARS-CoV-2 and their use in public health

Updated as at 23October 2020



TECHNICAL NOTE

Laboratory tests for SARS-CoV-2 and their use in public health

Updated as at 23 October 2020

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This document provides current interim guidance on the correct, rational and sustainable use of SARS-CoV-2 diagnostics and the ongoing criteria for choosing the most appropriate available test in each context.

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The responsibility for scientific and technical data lies with the authors, who declare that they do not have any conflict of interest.

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Acronyms

COVID-19	Corona Virus Disease – 2019
ECDC	European Centre for Disease Prevention and Control
INMI	*Istituto Nazionale Malattie Infettive* (National Institute for Infectious Diseases)
ISS	*Istituto Superiore di Sanità*
IVD	*In Vitro diagnostic medical Device*
LTCF	Long-Term Care Facilities
PD	Prevention Department
SARS-CoV-2	Severe Acute Respiratory Syndrome CoronaVirus 2
WHO	World Health Organization

Introduction and purpose of this document

This document arises from the need to provide guidance on the correct, rational and sustainable use of SARS-CoV-2 diagnostics and set out the criteria for choosing the most appropriate test in each setting.

This document summarizes the information available at the time of its publication and will be updated as new scientific evidence is generated.

The indications set forth are in line with the indications provided by the World Health Organization (WHO) for diagnostic products that target COVID-19 as reported in the document "Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0 "(1) published on September 28th, 2020 which describe the main characteristics of the tests for SARS-CoV-2, while emphasizing the need for them to meet not only specificity and sensitivity criteria, but also speed of execution so that they can be used in specific settings.

The European Centre for Disease Prevention and Control (ECDC) identifies five main objectives for testing:

- To control transmission;
- To monitor SARS-CoV-2 transmission rates and severity;
- To mitigate the impact of COVID-19 in healthcare and social care settings;
- To detect clusters or outbreaks in specific settings;
- To prevent the (re)introduction of the virus in areas where it has been eliminated.

This document constitutes one of the testing strategy tools that should be implemented as widely as possible across the entire Country.

Considerations on the use of diagnostic tests

Considering the evidence currently available, the epidemiological situation and the need to carry out tests in a fashion that is consistent with the different public health needs, it is of fundamental importance to choose tests that meet the different strategic objectives for SARS-CoV-2 ascertainment.

As reported by other experiences (2), high sensitivity and high specificity cannot be the only criteria considered when choosing the type of test to be used as part of a strategy that includes clinical diagnosis in a specific moment in time. One must also consider the possibility of repeating the test as part of surveillance activities using instruments that are sustainable and capable of detecting people when they are contagious.

Rapidity in diagnosing individuals with clinical and / or symptomatic signs and their contacts and isolating and quarantining them is essential in order to limit the transmission of the virus and control the outbreak.

Therefore, when choosing the tests to be adopted, various parameters appear to be important, namely:

- test execution times (a few hours for molecular tests, compared to 15-30 minutes for a rapid antigen test, for example);
- specialized personnel and dedicated instruments available only in a laboratory vs. small portable tests that can be used anywhere;
- cost of a retesting policy;
- transport of the samples versus on-site testing;
- acceptance by users for the invasiveness of the test;
- ease of sample collection;
- training required for collecting / processing samples;
- availability of reagents;
- stability of the samples (1).

Of critical importance is also the collection of data regarding the tests performed that can be used to analyse and evaluate the strategies adopted and the spread of the infection.

Implementation strategy

Here are some indications in support of the implementation strategy:

- **Suspected cases and confirmed cases**
  - The test is aimed at finding the virus in the context of clinical and epidemiological investigations of individuals with symptoms compatible with a SARS-CoV-2 infection, including close symptomatic contacts, and tests carried out to document the lack of infectivity in a previously diagnosed infection.
- **Asymptomatic close contacts**
  - Tests must be limited to close contacts of a confirmed case whether the test is prescribed at the beginning or at the end of the 10-day quarantine. In this regard, reference is to the Circular of the Ministry of Health no. 32850 of 12 October 2020 “COVID-19: indications for the duration and end of isolation and quarantine”.

- It is not recommended to prescribe diagnostic tests for contacts of close contacts of confirmed cases; if they are requested independently, the individuals must not be considered suspected or be subjected to any quarantine measures or reported to the Prevention Department (PD). Cases that are confirmed with the infection must always be communicated.
- If the request for diagnostic tests for close contacts, also in school settings, is made by a Primary Care Paediatrician or general practitioner (PLS/MMG), a copy of the request must always be sent to the competent Prevention Department (PD). At the same time, the individual is recommended to remain at home pending the test result.

- **Indications and general remarks**
  - Diagnostic analyses are to be carried out only in highly specialized Regional reference laboratories and in additional laboratories previously authorised by the Regions in accordance with the methods and procedures agreed upon with the National Reference Laboratory of the Istituto Superiore di Sanità.
  - The results of all the tests carried out, regardless of the laboratory involved, are to be forwarded to the Prevention Department (PD) in order to avoid retesting and above all allow the PD to implement public health measures that are in line with the test results.
  - Persons waiting for test results are to be quarantined. If the test results are positive, the PD will prescribe isolation for the person concerned and quarantine for close contacts. Although molecular tests are the gold standard for sensitivity and specificity, in many circumstances rapid antigen tests can be used which, in addition to being simpler and less expensive, provide results in less than half an hour, can also be performed at points of care and, if there is an epidemiological link, can help speed up the implementation of planned measures. For more details see the attached summary table.
  - At the testing sites (including drive-through testing centres) priority access needs to be defined for diagnostic tests prescribed for symptomatic subjects and asymptomatic close contacts of a confirmed case and to other envisaged categories. Non-priority tests could be performed in other settings (for example at accredited laboratories) using other methods to shorten the time for the collection of samples and the turnaround time for results which are essential for subsequent clinical investigations and for the adoption of public health actions.
  - In the case of rapid antigen tests, an adequate supply of instruments for swab reading is needed in order to optimize the ability to provide results in less than 30 minutes directly at the sampling site. Reading instruments are supplied together with the tests where fluorescence (IFA) or other systems are used, while the immunochromatographic tests do not require readers.
  - In light of the expected widespread use of rapid antigen tests, and given their simplicity, it might be possible to involve the network of Primary Care Paediatricians and General Practitioners in various organizational settings, including for community testing, subject to the adoption of adequate organizational, prevention and protection measures.
  - At present, rapid saliva tests (antigen or molecular) are being evaluated also in local settings. The data collected via the current pilot experiences are useful to define their indications for use in the near future.
  - It is noted that the definition of a SARS-CoV-2 “confirmed case” should be updated in order to define cases detected with rapid antigen tests but not confirmed using molecular tests. This is especially relevant in the presence of epidemiological links where positive rapid tests identify a “case” which then requires the rapid adoption of control measures.

- It is important that the data related to the validation of the various types of rapid antigen tests, including salivary tests, available in our country, is shared at the local / regional / national level in order to optimize the strategies for their use.
- It is emphasized that since the different types of tests are constantly evolving, validations carried out by the regulatory agencies of the G7 countries can be adopted for the purposes of their evaluation.

In the summary table, the use of the tests indicated as an alternative are to be considered in light of the epidemiological situation and the regional health organization.

Summary table of tests in the main settings

Setting	Type of test		Comments
	First choice	Alternative	
Suspected symptomatic case with or without an epidemiological link Symptomatic individuals are quarantined (e.g. close contacts of a confirmed case)	Molecular test on oro/nasopharyngeal swabs (**)	Rapid antigen test on oro/nasopharyngeal swabs or nasal swabs + confirmation of positive cases by molecular test (*)	Even though in this case the rapid test may have a higher degree of reliability because the individual is symptomatic (lower probability of a false negative), the molecular swab still remains the first choice
Mildly symptomatic individual without any epidemiological link Close contact of a confirmed case (in a school setting or workplace) who is asymptomatic, or who lives alone or who does not live with fragile or uncooperative people Asymptomatic individual coming from a Country at risk as per the current national law	Rapid antigen test on oro/nasopharyngeal swabs or nasal swabs without confirmation of positive cases by molecular test on oro/nasopharyngeal swabs	Molecular test on oro/nasopharyngeal swabs	In the case of contact tracing, the rapid antigen test can be a strategic and sustainable tool compared to traditional molecular tests where the molecular tests are outnumbered. Thanks to the rapidity of the results, infected individuals, if present, can be quickly identified and placed in isolation, thus limiting the spread of the infection in the community. In this case, the risk of false negative results is acceptable since it is offset by the speed of the response and by the fact that the test can be repeated if deemed necessary. More data are expected on the use of this test in asymptomatic individuals who have so far not been the target test recipients.
Close contact of a confirmed case who lives with or regularly sees fragile individuals at risk of complications or uncooperative individuals Asymptomatic individual needing hospitalization (***), joining large closed communities (e.g. LTCF, prisons, facilities for people with mental disorders, other) Screening of healthcare professionals / staff in high-risk settings	Molecular test on oro/nasopharyngeal swabs	Rapid antigen test on oro/nasopharyngeal swabs or nasal swabs + confirmation by molecular test on oro/nasopharyngeal swabs (*)	Molecular tests are highly sensitive in identifying positive individuals thus protecting fragile individuals at risk of complications and large communities at risk of clusters. Rapid antigen tests would be suitable in settings where positive individuals need to be rapidly identified and isolated in order to protect fragile individuals at risk of complications and to protect large closed communities.
Community screening (finding individuals with active infection in a large group of people) for public health reasons	Rapid antigen test on oro/nasopharyngeal swabs or nasal swabs + confirmation of positive cases without epidemiological links by molecular test on oro/nasopharyngeal swabs	Serological test + confirmation of positive cases by molecular test on oro/nasopharyngeal swabs (*)	Rapid antigen tests have the advantage of being quicker to perform and provide results faster than molecular tests. Serological tests can be processed rapidly in a laboratory or directly at point of care. Within the framework of a community strategy where it is necessary to immediately isolate a positive individual and quickly trace all possible contacts, the rapid antigen test does not need to be confirmed by a molecular test. The main limit of serological tests is that they may not detect the infection in its early stages nor when it is full-blown (antibody detection is time-dependent, poor predictive value of positive cases). Positive tests need to be confirmed by a molecular test. Hence the strategy for using serological tests in this setting is currently being assessed.

Setting	Type of test		Comments
	First choice	Alternative	
Individual in isolation while awaiting confirmation of full recovery	Molecular test on oro/nasopharyngeal swabs		
Asymptomatic contact in order to end quarantine after 10 days con	Molecular test on oro/nasopharyngeal swabs	Rapid nasal or oro/nasopharyngeal antigen test	
Asymptomatic individual who voluntarily takes the test; test required for work or travelling purposes (except specific provisions by countries of destination specifically requiring a molecular test) or for requests not linked to clinical or public health requirements that come under the previously described cases	Rapid antigen test on oro/nasopharyngeal swabs + confirmation of positive cases by molecular test on oro/nasopharyngeal swabs	Molecular test on oro/nasopharyngeal or nasal swabs	Tests performed within Regional facilities (paid by the users or as part of a public health plan)

(*) Considered “confirmed” in case of epidemiological link +positive rapid antigen test and hence control measures are adopted

(**) Molecular test also using the “raw” technique

(***) In case of unscheduled hospitalization such as access to the emergency room, the rapid test could be the first choice, depending on the organization of the health facility, in order to direct the patient in the correct path.

NOTE The rapid saliva antigen tests, currently being tested, will be considered as an alternative to the rapid antigen tests on oral-pharyngeal or nasal swabs if the validation and pilot experiences currently underway in Italy provide results that suggest they can be used also in public health settings. It should be emphasized that the saliva collection systems of the “Salivette” type do not appear to be adequate for the time being given the modality of the test and because uncooperative individuals run the risk of swallowing the collection device.

APPENDIX

Tests currently available for public health purposes

The European Commission has published a document on the data available for commercial CE-marked IVD tests (3) which include analytical sensitivity and specificity, clinical sensitivity and specificity, and is collecting, in a searchable database, the data of the manufacturers of the CE-marked commercial IVD tests and reviewing internally developed laboratory tests backed by performance data published in scientific journals(4). These lists are constantly updated.

Currently the tests can be divided into three large groups: molecular swabs, rapid antigen swabs, serological tests.

Molecular swab test

This is a molecular reverse transcription (rt) -Real Time PCR investigation for the detection of the SARS-CoV-2 virus genome (RNA) in a biological sample. With this method one or more target genes of the virus present in the biological sample can be identified in a highly specific and sensitive manner and the initial concentration of the target sequence can be measured in real time. Albeit with qualitative indications as described and suggested by the international bodies of reference, with the rt-Real Time PCR assays an amplification curve can be obtained whose Cycle Threshold (CT) is inversely proportional to the quantity of the gene target as initial template present in the sample. Therefore, according to this principle, the greater the number of "template molecules" present at the start of the reaction, the lower the number of cycles required to reach a certain CT value. Conversely, a high CT value, obtained after numerous amplification cycles, indicates a low amount of initial gene target and therefore of viral genome.

RT-Real Time PCR is the "gold standard" for diagnosing COVID-19. The detection of SARS-CoV-2 viral RNA performed in the laboratory from clinical samples (usually nasopharyngeal or oropharyngeal swabs) remains the international assay of reference for sensitivity and specificity and is able to detect the pathogen even at low viral loads in symptomatic, pre-symptomatic and asymptomatic subjects. Due to the complexity of the method, the detection of SARS-CoV-2 is performed exclusively in specialized laboratories with expert health workers (5). The results can be obtained in a minimum of 3-5 hours but for organizational and logistics reasons the results can take 1-2 days. It is worth recalling that for the purposes of entering cases into the integrated COVID-19 surveillance system, coordinated by ISS, only the positive results obtained through rt-Real Time PCR by regional laboratories of reference, or by the laboratories identified / authorized by them, are considered (https://www.epicentro.iss.it/coronavirus/sars-cov-2-sorveglianza). In addition, instead of extracting and purifying the RNA from the biological sample, the molecular test analysis can also be carried out by using the heat inactivation technique (95°C/98°C) ("raw"). This simplifies, maximizes and speeds up the first step of the analysis itself, especially in conditions where the laboratories are under excessive stress due to the increase in swabs samples to be processed. The risk of a loss in sensitivity is minimal and present only for high numbers of PCR amplification cycles, as reported by international scientific papers (6-7). However, sensitivity is superior to that of the antigen test.

Rapid antigen test (by means of nasal, oropharyngeal, saliva swabs)

In recent months, new types of tests have been developed that promise to deliver results faster (30-60 minutes), at lower cost and without the need for specialized personnel (8). These are rapid antigen tests, potentially useful tools especially for screening purposes. Similarly, to molecular tests, antigen assays are direct diagnostic tests, i.e. they directly detect the presence of the virus in the clinical sample, unlike serological tests which are indirect diagnostic tests, i.e. they detect the presence of specific antibodies that indirectly indicate a previous or current infection. Unlike molecular tests, however, antigen tests detect the presence of the virus not through its nucleic acid but through its proteins (antigens). These tests contain

specific antibodies as substrate that bind to the viral antigens of SARS-CoV-2 and the result of the antigen-antibody reaction is directly visible to the naked eye or can be read by a simple device at the "point of care" without requiring a laboratory for processing. In the US 3 rapid antigen tests have been cleared by the FDA. In Europe, numerous tests have already obtained the CE mark and several diagnostic companies are working to obtain the appropriate regulatory approval so as to place the tests on the market with a sufficient degree of reliability (9).

The antigen tests are qualitative (yes / no) and, through polyclonal or monoclonal antibodies, they spot specific peptides (protein portions) of the S (Spike) or N (nucleocapsid) protein present on the viral surface of SARS-CoV-2.

The test may be negative if the concentration of antigens is lower than the detection threshold of the test (e.g. if the sample was taken too early compared to the hypothetical moment of exposure) or if the collection, transportation or storage of the sample was undermined. For this reason, the manufacturers of these kits highlight that a negative test result does not rule out the possibility of a SARS-CoV-2 infection, and where the suspicion of a COVID-19 infection is strong, a negative sample should be confirmed by molecular testing. Molecular tests appear to have greater sensitivity before the onset of symptoms, while in the initial phase, immediately following the onset of infection, rapid antigen and molecular tests have similar sensitivity, making it useful to use the former as well (2). In addition, the rapid antigen test can be used for the identification of the asymptomatic contacts of confirmed cases, although this type of test is not specifically authorized for this use, as asymptomatic cases have been found to present viral loads similar to symptomatic cases (10).

Unfortunately, to date there are not enough published studies that, with regard to specific settings and massive numbers of cases, can provide indications about the sensitivity and specificity of these rapid tests. At present, the available data of the various tests for these parameters are those declared by the manufacturer: 70-86% for sensitivity and 95-97% for specificity (11-14).

The Foundation for Innovative New Diagnostics (FIND) (https://www.finddx.org/covid-19/pipeline/?section=show-all#diag_tab) has created a rapidly evolving online platform that brings together a vast series of assays for SARS-CoV-2 ranging from the early stages of development to full regulatory approval.

Regarding saliva swab tests, the detection device is the same, but changing the sample analysed can change the sensitivity and specificity characteristics of the test. In conclusion, the sensitivity and specificity of these rapid antigen tests need to be evaluated for their predictive value throughout the course of their technological development.

It is important to share the validation data about the various types of rapid antigen tests, including salivary tests, available in our Country at a the local / regional / national levels.

Serological tests

Serological tests detect cases of exposure to the SARS-COV-2 virus but are unable to confirm whether or not an infection is in progress. For this, in case of a positive test, a molecular swab test is required for confirmation. The Circular of the Ministry of Health no. 16106 of the 9th of May 2020, reiterates that "the quality and reliability of a test depend in particular on the two characteristics of specificity and sensitivity, and therefore, although there are no legal obligations in relation to them, the use of CLIA and / or ELISA type tests with a specificity of at least 95% and a sensitivity of at least 90% is strongly recommended, in order to reduce the number of false positive and false negative results. Below these thresholds, the reliability of results is not adequate for the purposes for which the tests are performed".

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