

Recommendations for specimen collection for detection and diagnosis of COVID-19

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ABSTRACT

Introduction: The spread of SARS-CoV-2 has caused a global public health crisis (pandemic). One of the most important measures to control the transmission chain of the new coronavirus is to identify those infected through laboratory testing. Objective: Synthesize the recommendations for the specimen collection for detection and diagnosis of COVID-19. Methods: This is an integrative review, considering the publications of the following databases: PubMed and Google Academic from January 2020. Results: 468 publications were identified, 20 of which were considered eligible. The publications recommend that technical training for specimen collection and careful observation of infection prevention protocols are fundamental. This manuscript highlights the steps for specimen collection as materials for collection, storage, transportation, individual protection, and laboratory analysis of samples. Currently, the Reverse Transcription - Polymerase Chain Reaction test is the recommended and gold standard method of identifying COVID-19 cases. Serological tests play an important role in research and surveillance. Conclusion: In summary, the documents ensure that the RT-PCR is the gold standard for SARS-CoV-2 detection and recommend standardization of collection and conditioning methods to avoid errors related to the collection and false negative results.

Keywords: specimen handling; coronavirus infections; protein corona; pandemics; diagnosis.

INTRODUCTION

The emergence and spread of the new coronavirus, or Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which leads to the development of Coronavirus Disease-2019 (COVID-19), has caused a crisis in public health worldwide¹. The COVID-19 pandemic was established by the World Health Organization (WHO) on March 11, 2020. There are about 104,765,377 reported cases of coronavirus, 76,436,911 of which were recovered, and 2,273,516 deaths reported worldwide on 03 February 2021. On the same date, in Brazil, 9,296,945 were reported infected, 226,666 deaths, and 8,160,929 recovered². Identifying the infected through laboratory testing and breaking the chain of transmission of the virus, isolating infected people for at least 14 days, and tracking their contacts is one of the most important measures of disease control^{3,4}.

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This is an open access article distributed under the terms of the Creative Commons Attribution License © 2021 Dall'Agnol et al. The molecular test, real-time polymerase chain reaction (RT-PCR), is the method currently recommended by WHO for the identification of cases of COVID-19. Serological tests play an important role in research and surveillance but are not recommended for the detection and diagnosis of COVID-19, as they detect the immune response. Rapid tests (IgM/IgG) can help map the population that has already contracted or been exposed to SARS-CoV-2. However, the rapid test has no diagnostic function^{1,3-9}. It is important to note that the rate of false negative results of IgM antibody tests (detection of COVID-19 in the acute phase) registered by the National Health Surveillance Agency (ANVISA) ranged from 10% to 44%¹⁰.

Currently, the nasopharyngeal test (swab) by RT-PCR is considered the gold standard for the detection of the new coronavirus and other pathogens of the upper respiratory tract^{1,3-8}. However, the worldwide shortage of inputs for RT-PCR tests, such as the lack of swabs, reagents, and means of transport, has impacted the capacity and availability of these tests. In order to circumvent the scarcity of inputs, new methods are being developed for the diagnosis of SARS-CoV-2^{11,12}.

One of the tests developed is the saliva sample self-collection. This method proposes to reduce the risk of exposure of health professionals, to reduce the time of analysis and the cost of the procedure. In this context, research centers such as at the University of São Paulo, in Brazil, at Yale University, in the United States, other institutions located in Brazil, such as the Sociedade Beneficente Israelita Brasileira and the Hospital Sírio-Libanês, work on developing tests that can meet the needs cited. Yale University presented, in August 2020, the development of a test for the new salivabased coronavirus called SalivaDirect¹³⁻¹⁸.

This integrative bibliographic review is justified by the worldwide spread of the new coronavirus and the need to verify the best conduct in terms of collection, input logistics, professional training, and scope of tests, especially in developing countries.

This study aimed to synthesize the recommendations for collecting samples for the detection and diagnosis of COVID-19.

METHODS

This is an integrative bibliographic review of documents published worldwide. The integrative review enables the identification and analysis of scientific evidence when scientific knowledge is not yet consolidated¹⁹.

The question of the integrative review expressed by: what are the collection methods for the diagnosis of the new coronavirus determined? And the complementary questions: who is involved in the collection, use of personal protective equipment (PPE), inputs used, conditions for transportation. The following search strategies were used: the definition of words and Medical Subject Headings and search in the databases on Google Scholar and PubMed. The word combinations (COVID OR coronavirus OR SARS CoV) AND (collection OR test) AND (laboratory OR diagnosis) were also used.

The inclusion criteria for the documents were: to be published in Portuguese, English, or Spanish; have been conducted on humans; published as of January 2020. Exclusion criteria were: publications that did not answer the review question and publications that did not address laboratory tests for confirmatory diagnosis for SARS-CoV-2.

The treatment of the bibliographic data was carried out with fairness, integrity, impartiality, and respect to the original authors of the publications that are part of this study, according to the recommendations contained in the Resolution of the National Health Council 466/2012²⁰.

The selection of documents was carried out in two stages (Figure 1): In the first stage, one of the reviewers selected the documents in accordance with the inclusion and exclusion criteria; in the second stage, two reviewers performed the reading in full



Figure 1: Flowchart of the integrative bibliographic review.

and independently the information that answered the question of this review was collected¹⁹. The collected data were registered in the Excel Software, with the purpose of organizing the database.

RESULTS AND DISCUSSION

296 articles were identified in Pubmed and 172 documents in Google Scholar. Twenty documents were considered eligible for this study, six articles in Pubmed and 14 documents in Google Scholar. The materials found have references to WHO and the Centers for Disease Control and Prevention (CDC)^{1,3-5,21-26}.

The publications found emphasize that care in the stages of collection, storage, and transport is essential since such procedures performed inappropriately can generate tests with false results and a high risk of contamination by professionals. For this reason, they recommend technical training for health professionals to carry out the collection of samples following international protocols and technical notes from the surveillance agencies in their country regarding the prevention of infection, precautions, storage, transport, and laboratory analysis^{3-5,7-9,11-15,17,18,21-26}.

To carry out the collection and sending of samples to the laboratories, the standard operating procedures of each institution must be observed, guided by the WHO, CDC, and by Organs national health surveillance agencies. These procedures must occur with adequate technical and human resources, and the professionals involved are trained to collect, store, transport, and analyze the samples (Table 1). All samples collected for laboratory diagnosis should be considered potentially infectious^{3-7,21,24}. Epidemiological data collected in several countries highlight that mass testing is essential to combat the new coronavirus. The WHO strongly recommends mass testing through RT-PCR tests to identify those infected, isolating people for at least 14 days, and tracking their contacts, are important measures to control the disease. In addition to mass testing and social isolation, hand hygiene and the use of masks are added. It is also highlighted the need to implement these measures in order to guarantee the greatest impact on public health by reducing transmission using available resources. Mass testing, applied to individuals who show symptoms and periodically to health workers, is still considered one of the major challenges in combating the new coronavirus³.

It is recommended that the collection for the diagnosis and detection of COVID-19 be carried out with the monitoring of a health professional immediately after consultation at a health service. Therefore, all health professionals involved in the collection must use PPE, such as: N95 mask or with greater filtration efficiency, hair cap, double gloves, eye protector (facial or glasses) and protective clothing and/or apron; and, in cases of exposure to blood, body fluids (secretions or excretions) from individuals it is recommended to use waterproof protectors and boots^{4,5,11,12,21,22,24,25}.

Types of samples

The nasopharyngeal swab is a widely used sample⁹. Regarding the samples, it is recommended that these be collected through the upper respiratory tract, including samples with nasopharyngeal, oropharyngeal swabs, aspirated and nasopharyngeal

Type of sample	Material for collection	Storage temperature*	Responsible for collection	Individual protection equipment
Nasopharyngeal and/or oropharyngeal secretion 5,7,22-26	Sterile Swab	2°C- 8°C (5 days) 4°C (48h)	Healthcare professional	Mandatory
Nasal secretion or nasal mid7, 26	Sterile Swab	2°C- 8°C 4°C (48h)	Health or supervised professional**	Mandatory
Aspirated/tracheal, nasal and nasopharyngeal lavage ^{11,22,24,26}	Sterile Collector	2°C- 8°C (48h) 4°C (48h)	Healthcare professional	Mandatory
Aspirated/washed, tracheal, bronchoalveolar ^{11,22,24,26}	Sterile Collector	2°C- 8°C(48h) 4°C (48h)	Healthcare professional	Mandatory
Sputum 5,26	Sterile Collector	2°C- 8°C (48h) 4°C (48h)	Health or supervised professional**	Mandatory
Saliva ¹³⁻¹⁸	Collector tube	Room temperature	Self-collected supervised or healthcare professional**	Mandatory or not required for self-collection
Blood ^{25,26}	Collector tube	2°C- 8°C (5 days) 4°C (48h)	Healthcare professional	Mandatory
Feces ^{25,26}	Collector or swab	2°C- 8°C (5 days) 4°C (48h)	Health or supervised professional**	Mandatory
Tissue/organs (autopsy)26	Sterile container	2°C- 8°C (24h) 4°C (48h)	Healthcare professional	Mandatory
Urine ^{25,26}	Urine collector	2°C- 8°C (5 days) 4°C (48h)	Health or supervised professional**	Mandatory

Table 1: Recommendations for collecting samples for detection and diagnosis of the new coronavirus, 2020.

* Sample that cannot be analyzed in the period must be transported at -70°C on dry ice. In the absence of these conditions, it can also be stored in a -20°C refrigerator. ** Supervised by a healthcare professional. lavage. Swab samples are the most frequently sent to laboratories for analysis. In case of impossibility to perform the procedure for the upper tract or in severe cases, samples of the lower respiratory tract such as sputum, tracheal aspirate, and bronchoalveolar lavage, pleural fluid, and lung biopsy can be collected or, depending on clinical needs, they can be considered collection of stool, serum and urine samples^{11,22,24,26}.

The swab used to collect the nasopharynx and oropharynx must be made of synthetic fiber with plastic rods. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate the new coronavirus and inhibit the RT-PCR test. The recommendation is to collect only via nasopharyngeal, although samples from the oropharyngeal route remain an acceptable type of sample. If nasopharyngeal and oropharyngeal swabs are collected, they should be combined in a single tube to maximize the sensitivity of the RT-PCR test and limit the use of inputs^{57,23,24,26}.

For collection (Figure 2), use a sterile swab for secretion in the upper airways, nasopharyngeal or oropharyngeal or, still, nasal, to be performed by the health professional or proceed to supervised self-collection on the spot, immediately placed in a tube with sterile material for maintaining the sample in 2-3 ml of saline^{5,7,23-25}.

The recommended technique for collecting nasopharyngeal secretion is to insert the sterile swab with a flexible plastic shaft through the nostril parallel to the palate (not upwards) until resistance is found or the distance is equivalent to that of the ear to the individual's nostril, indicating contact with the nasopharynx. Rub and roll the swab gently for a few seconds to absorb and slowly remove the sample to be collected. The same swab can be used in the other nostril if there is resistance due to the presence of deviated septum^{57,22,23,26}. It is recommended to collect a set of three swabs (two nasopharyngeal and one oropharyngeal) and insert the three swabs in the same bottle containing saline²⁴.

Samples from the lower respiratory tract include: sputum, tracheal aspirate, bronchoalveolar lavage, pleural fluid, and lung biopsy and should be limited to individuals with severe hospitalized



Figure 2: Nasopharyngeal sample collection.

conditions and fatal cases. The samples are collected in sterile collectors and before starting treatment with antimicrobials. For sputum collection, the individual must rinse his mouth with water and expectorate the sputum in a sterile container with a deep cough^{5,26}.

The nasopharyngeal aspirate collector must be coupled to a No. 6 urethral probe with only one hole at the end to obtain secretion through the hospital wall vacuum. The probe must be inserted into the nostril until it reaches the nasopharynx (6 to 8 cm), and then the vacuum is applied, aspirating the secretion. Alternate the collection in the nostrils until a sufficient volume is obtained, approximately 1 mL of nasopharyngeal aspirate. The vacuum must be activated after the probe is in the nasopharynx. After obtaining the secretion, aspirate the viral transport medium into the collector with the same probe. Discard the probe in infectious waste and seal the collector orifice²⁴.

Collection of post-mortem samples

Serum samples for fatal, post-mortem, and autopsy situations should be obtained by collecting 5-10ml of blood in a collection tube with an anticoagulant. In order to avoid contamination, samples of tissues/organs are collected with an aseptic technique as soon as possible in all fatal cases of the main tissues and organs (lung and other organs with suspected involvement). These tissue/organ samples must be stored in sterile containers containing saline solution. The urine sample must be collected in a sterile 10-20ml collector. Fecal samples must be collected in 10-20ml or 10g in a clean, dry, and leak-proof container, or an anal swab can be collected. All samples must be identified with the individual's name, using the individual's identity number or otherwise provided by the state, even the laboratory's requisition number with the type of sample and date of collection and sample identification collected²⁶.

Sample storage

The samples must remain in thermal storage (refrigerator) of 2°C to 8°C (4°C on average) or, still, at room temperature, depending on the type of sample (nasopharyngeal and oropharyngeal secretion, blood, feces, and urine) in the period of 24 hours up to 5 days. However, if there is a delay in shipment to the laboratory or those samples that cannot be analyzed in that period, they must be transported at -70°C until shipment, ensuring that they maintain the temperature in dry ice and in UN3373 packaging, according to the Association's Norms. International Air Transport (IATA). In the absence of these conditions, the samples can still be temporarily stored in refrigerators with a temperature below -20°C. For transportation, the use of ice and a thermometer is recommended to maintain and control the temperature^{3,7,24,26}. Furthermore, the storage boxes must be closed/sealed and contain only the samples. The box must also be identified (Coronavirus/COVID; Influenza). Requests must be posted on the outside of the box²⁴.

In relation to post-mortem care, to health professionals, it is recommended as a good biosafety practice, immediate communication to the state health department, in cases of people who have died due to suspicion or confirmation by COVID-19. Special attention is recommended in the use of contaminated sharps, with appropriate disposal, considering the use of a record book with information regarding the identification of those involved in the procedures with names, dates, and activities of all workers who participated in the autopsy and cleaning the autopsy room²⁵.

Tests based on saliva

Currently, there is a difference in how countries are dealing with SARS-CoV-2 diagnostic tests due to supply limitations. Home testing and drive-thru strategies can allow rapid testing to increase and thus can alleviate supply chain problems^{11,12}. SARS-CoV-2 virus tests with saliva recently received the United States Food and Drug Administration (FDA) Emergency Use Authorization for home sample collection¹⁵.

The saliva sticker (saliva that naturally accumulates in the mouth) is an alternative for obtaining invasive collections, as it allows easier and safer collection for the diagnosis of COVID-19. In addition, the test can be maximized without the need for expensive collection tubes, extraction kits, and even cost savings on the necessary RT-PCR reagents per sample. RT-PCR and RT-LAMP (Reverse Transcription Loop-Mediated Isothermal Amplification) showed sufficient sensitivities in clinical use to be used¹¹⁻¹⁸.

In Brazil, the Mendelics laboratory, in partnership with the Hospital Sírio-Libanês, the so-called PareCovid, detects the virus through saliva in 60 minutes. The first difference is the self-collection process, in a painless and non-invasive way, since the person himself can collect 2 ml of his saliva in a test tube, and the sample stability is up to 3 days at room temperature. The saliva test showed sensitivity comparable to nasopharyngeal swabs in the detection of other respiratory pathogens¹³⁻¹⁸.

Conclusion

This integrative bibliographic review made it possible to summarize the recommendations for collecting samples for the detection and diagnosis of COVID-19. Although it is a relevant topic today, the lack of consolidated knowledge on the subject made it impossible to include experimental and quasi-experimental research documents.

The RT-PCR diagnostic test is considered the gold standard test for the detection of SARS-CoV-2. Although serological tests are not suitable for diagnosis, they are important for defining epidemiological issues and identifying immune individuals, including the rate of infected individuals in the population.

Sample collection and packaging are important steps for the laboratory diagnosis of COVID-19 in order to avoid errors related to the collection and false negative results. The new studies suggest selfsampling of upper respiratory tract samples for diagnostic purposes when possible, as it may increase the coverage rate of the molecular test, reduce the shortage of PPE and reduce the SARS-CoV-2 infection rate of healthcare professionals involved in the collection.

Depending on the type of sample, it must be collected by a health professional or supervised by a professional or self-collection. Regardless of the technique used, the recommendation is that all health workers should use PPE, according to the local infection control protocol of the health institution and according to the health surveillance rules of each country.

The limitations of this study are related to the method used, such as databases, language, period of study, non-systematic review, so as not to allow the analysis of all publications to be exhausted, with the recommendations presented being considered as best approaches so far.

In summary, the studies propose the development and standardization of faster testing methods and pointing out the need to organize the health system, with the aim of advancing health care in the face of viral infections.

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