Biological Samples - Collection, Packaging and Transport
COVID-19 Context

The laboratory's responsibility in issuing reliable test results is very great, as it can directly impact the care decision, be it a diagnosis, a definition of prognosis or therapeutic monitoring. The literature shows that 70% of medical decisions are made based on a laboratory exam.

Studies carried out in different health reference centers, related to errors in laboratories, found that approximately 60 to 90% of laboratory errors found are a consequence of the lack of standardization in the pre-analytical phase. Therefore, it is extremely important to implement more rigorous methodologies for detecting, classifying, and reducing these errors.

According to the World Health Organization (WHO), the decision to test a patient must be based on clinical criteria and epidemiological factors. In the current pandemic facing the world, testing for COVID-19 in asymptomatic or mildly symptomatic individuals should be considered in cases of individuals who have had positive contact with SARS-CoV-2 patients.

Laboratory tests

The WHO recommends that during the pandemic, all patients who show suspicious signs and symptoms for COVID-19 should be tested for the SARS-CoV-2 virus. The test does not preclude laboratory research for other pathogens that generate community pneumonia, since co-infections may exist, however, even if another respiratory pathogen is found, the test for COVID-19 must be performed.

The correct performance of collection procedures, storage and transport of samples is related to the quality of the results provided by the laboratory, aiding decision making and patient care.

Sample collection

Health units must ensure good communication with laboratory units. It is recommended that the laboratories have specific flows for identifying patients requesting tests for SARS-CoV2 research.

Collections should be carried out in accordance with institutional protocols and the recommendations of health regulatory agencies.

PPE - Personal Protective Equipment

During collection, the use of personal protective equipment (PPE) is mandatory:

- Procedure gloves
- Cloaks
- N95 mask
- Caps
- Eye protection device.

Sample identification

All tubes must be identified with the patient's data and other information necessary for their traceability. In addition, it is important to define a specific

identifier (eg label, pen color, etc.) to differentiate the tubes collected from patients suspected of SARS-CoV-2 contamination from other materials.

Biological fluids that can be tested

The Brazilian Society of Clinical Analyzes (SBAC) published a technical report in March 2020, which reports that both upper respiratory tract fluids (nasopharyngeal and oropharyngeal secretion, nasopharyngeal aspirate / lavage, nasal aspirate), and those of the lower respiratory tract (sputum , bronchoalveolar lavage, tracheal aspirate) can be used and their choice will depend on the resources available and the patient's clinical status.

The CDC recommends the simultaneous collection and testing of upper respiratory tract samples (nasopharynx AND oropharynx swabs) for all cases, and lower respiratory tract sample (sputum, when possible) in cases of patients with productive cough.

- Nasopharyngeal secretion: Use only synthetic fiber swab with plastic rod. Perform the institutionalized swab collection procedure. Always use two swabs, one for each nostril. After collection, immediately insert them into the vial containing the virus transport medium.
- Oropharyngeal secretion: Use only synthetic fiber swab with plastic rod. Perform the institutionalized swab collection procedure. After collection, immediately insert them into the flask containing virus transport means, do not use the same tube used for the nasopharynx.

ATTENTION: Never use a swab with wooden rods or containing calcium alginate, as they may contain substances that inactivate some viruses and inhibit the PCR test.

- Aspirated or nasopharyngeal lavage, bronchoalveolar lavage, or tracheal aspirate: Collect 2 to 3 mL of the material in a leak-proof container, with screw cap, dry and sterile.
- Sputum: Sputum induction is not recommended, so this sample should only be collected from patients with productive cough. Ask the patient to rinse his mouth with water and then cough hard and directly into the collection container. This collection must be done in an appropriate environment that allows the isolation of the patient during its execution due to the production of aerosol. Collect 2 to 3 mL of the material in a leak-proof container, with screw cap, dry and sterile.

Transport

The CDC and WHO reinforce that the samples collected for virus detection must reach the laboratory as soon as possible, and that the proper and safe handling of these samples during transport is essential.

- Patient samples from suspected or confirmed cases should be transported as UN3373 “Biological. Substance, category B ”.
- Viral or isolated cultures must be transported as Category A, UN2814, “infectious substance that affects humans”.

The RDC nº 20 of ANVISA 2014, which regulates the transport of human biological material, specifies in detail the appropriate ways of packaging and transporting biological materials of categories A and B, with safety and maintaining the stability of the samples.

Storage

The samples can be stored refrigerated or frozen and, if necessary, stored on dry ice during transport. The freezing and thawing of the samples, repeatedly, can lead to the loss of the collected material.

Sample type	Collection material	Storage temperature for analysis within 72 hours	Storage temperature for analysis after 72 hours
Nasopharyngeal and oropharyngeal fluid	Synthetic fiber swab with plastic rod and vial containing means of transport for viruses	2-8 °C	–70 °C

Bronchoalveolar lavage	Leak-proof container with screw cap, dry and sterile.	2-8 °C	–70 °C
Sputum	Leak-proof container with screw cap, dry and sterile.	2-8 °C	–70 °C
Biopsy or autopsy tissue, including from the lung	Sterile saline container	2-8 °C	–70 °C
Blood sample	Collection tube	2-8 °C	–70 °C

Sample type	Collection material	Storage temperature for analysis within 48 hours	Storage temperature for analysis after 48 hours
Endotracheal suction, washing / nasopharyngeal aspiration.	Leak-proof container with screw cap, dry and sterile.	2-8 °C	–70 °C
Pleural fluid.	Leak-proof container with screw cap, dry and sterile.	2-8 °C	–70 °C

Source: CDC e WHO (references 1 e 8).

Laboratory methods: CRP and Serology for COVID-19

Like the coronaviruses responsible for SARS and MERS, the SARS-CoV-2 virus has already been detected in feces, however, it is unknown how long it survives, which is why there is no defined protocol for its research on this material.

- CRP

According to WHO and CDC, the gold standard for laboratory diagnosis of COVID-19 is the reverse transcriptase reaction, followed by the polymerase chain reaction (RT-CRP) for samples collected in the upper or lower respiratory tract.

The serological test identifies the presence of specific antibodies and proteins produced in response to infection. These antibodies can be found in the blood and other tissues of people who tested positive for infection using the RT-CRP method. The results of the antibody tests are important in detecting infections with few or no symptoms.

According to the WHO, the sensitivity of the test varies according to the origin of the sample of respiratory secretion, being 93% for bronchoalveolar lavage, 63% for nasal swab and 32% for pharyngeal swab (2).

- Rapid tests

The WHO recommends the performance of a rapid test (using the methodology called immunochromatography or chemiluminescent immunoassay) in suspected patients for COVID-19, as it assists in clinical management and outbreak control, and points out that it should always be performed with the monitoring of a professional specialized in clinical analysis.

SBAC published a technical report on the analysis of the package insert for the Rapid Test Kits of the main manufacturers operating in the Brazilian market, currently, and reached the following information:

⇒ Sample types (serum, plasma, whole blood) - Analyze the instructions for the reagent used.

⇒ Specificity: The specificity cited by KIT manufacturers for IgM antibodies varies between 95% to 96%, according to the manufacturer. For IgG type antibodies it ranges from 95% to 98%.

⇒ Sensitivity: Regarding the sensitivity for IgM antibodies, all manufacturers report 85% and for IgG type antibodies it ranges from 95% to 100%.

⇒ Cross Reactions: Research reports testing cross-reactions with positive samples for anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-adenovirus, anti-syphilis, anti-H.Pylori, anti-HIV, anti- HCV and HBsA, and that the results showed no cross-reactivity.

⇒ Test limitations: one of the manufacturers claims that the hematocrit level of the whole blood tested can affect the test result and states that the sample hematocrit value must be between 25% and 65% in order to obtain accurate results.

⇒ Interfering: one of the manufacturers states that the tests carried out did not show any interference from the substances mentioned below up to the indicated concentrations: Triglycerides up to 50 mg / dl; Hemoglobin up to 1000 mg / dl, Ascorbic acid up to 20 mg / dl, Bilirubin up to 60 mg / dl, Cholesterol up to 6 mmol / L (approximately 232 mg / dl). None of the inserts mentioned that positive and negative controls will be provided with the reagents.

⇒ Sample stability: serum or plasma: 2º to 8ºC up to 7 days; storage below -20ºC is quoted by one of the manufacturers, which however does not specify the maximum time. Whole blood obtained by venipuncture: 2º to 8ºC for up to 48 hours. Whole blood obtained by capillary puncture: analysis must be immediate

Note: SBAC recommends that, as some sets of reagents for serological tests were authorized by Anvisa on an emergency basis, due to the seriousness of the situation and the need to expand the testing of the population, laboratories carry out the validation of these reagents, since few studies managed to be published so far.

Remote laboratory tests (rapid tests) were developed to be performed on portable equipment, which can be allocated in different locations, in accordance with Resolution No. 302 of October 13, 2005, which provides for the Technical Regulation for the operation of the laboratories and must be linked to a clinical laboratory, collection point, or outpatient or hospital public health service.

This same resolution defines that the technician in charge of the clinical analysis laboratory is responsible for all exams performed remotely by this methodology in hospitals, ambulatory units, day hospital, collection point, including laboratory collection at home or in a mobile unit. The choice of the analytical methodology of the tests, the training of professionals for the collection, storage, execution of the test and release of the report is the responsibility of this professional.

At each location where this test is performed, guidelines should be available for:

- Acceptable sample type,
- Sample stabilization time;
- Sample restriction;
- Diagnostic and technical limitations of the test (sensitivity, specificity and interfering factors in the sample).

It is mandatory item in the issuance of the provisional report the complete identification of the patient, time of collection and performance of the test. The final report can only be signed by a qualified professional linked to the Technical Responsible (TR) of a clinical laboratory.

Results

One or more negative results do not rule out the possibility of infection with the new coronavirus (SARS-CoV-2). Many factors can lead to a negative result in an infected individual, including:

- Low quality of the sample, containing little material from the patient.
- Sample collected late or very early in the infection.
- Specimen was not handled, stored and shipped properly.
- Technical reasons inherent to the test (viral mutation or PCR inhibition).

In cases with a high index of suspicion and negative results, especially when only upper airway samples were collected, it is recommended to test a sample collected from the lower airway.

Biosafety

According to the WHO, laboratories that carry out tests for the new coronavirus SARS-CoV-2 should reinforce internal security:

- Any tests for the presence of SARS-CoV-2 must be carried out in properly equipped laboratories and by a team previously trained in the relevant technical and safety procedures.
- Each laboratory must perform a local (institutional) risk assessment to ensure a safe place for adequate sample storage and testing for SARS-CoV-2.
- Laboratory biosafety guidelines must be followed at all stages.
- The handling and processing of samples of cases with suspected or confirmed SARS-CoV-2 infection for additional laboratory tests, such as hematology or blood gas analysis, must follow specific guidelines to avoid contamination.
- The handling of material with high concentrations of live viruses (virus isolation or neutralization assays) or large volumes of infectious materials should be performed only by appropriately trained people.
- It is recommended to perform the disinfection with a solution to be defined by the hospital infection service of the vial and wrapping of the material of patients suspected or confirmed by Covid 19, before sending it to the laboratory.
- Initial processing (before inactivation) of all samples, including those for sequencing and NAAT, must take place in a biological safety cabinet, properly maintained, and validated or in a primary containment device.

- All technical procedures must be performed in order to minimize the generation of aerosols and droplets.
- The appropriate Personal Protective Equipment (PPE), as determined by the institutional risk assessment, must be used by all laboratory staff handling these samples.
- All material, equipment, benches, tubes, among others, must be disinfected with appropriate chemical products (0.1% sodium hypochlorite or 70% ethanol, for at least 1 minute of exposure).

References

1 - World Health Organization (WHO) - Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Interim guidance 2 March 2020 - WHO/COVID-19/laboratory/2020.4 - https://apps.who.int/iris/bitstream/handle/10665/331329/WHO-COVID-19-laboratory-2020.4-eng.pdf

2 - World Health Organization (WHO) - Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV) Interim guidance 12 February 2020 - WHO/WPE/GIH/2020.1 https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspected-20200125

3 - POSICIONAMENTO OFICIAL DA SOCIEDADE BRASILEIRA DE PATOLOGIA CLÍNICA/MEDICINA LABORATORIAL (SBPC/ML) - DIAGNÓSTICO LABORATORIAL DA INFECÇÃO PELO NOVO CORONAVÍRUS (COVID-19), 2020. http://www.sbpc.org.br/wp-content/uploads/2020/02/DiagnosticoLaboratorial-DaInfeccaoPeloNovoCoronavirus.pdf

4 – Instituto de Gestão Estratégica Em Saúde do Distrito Federal (IGESDF) - DIRETORIA DE ENSINO, PESQUISA E INOVAÇÃO NÚCLEO DE AVALIAÇÃO DE TECNOLOGIAS EM SAÚDE – NOTA TÉCNICA – TESTE DIAGNÓSTICO PARA COVID-19 https://igesdf.org.br/noticia/nota-tecnica-teste-diagnostico-para-covid-19/

5 - RESOLUÇÃO DE DIRETORIA COLEGIADA – RDC Nº 302, DE 13 DE OUTUBRO DE 2005 - Dispõe sobre Regulamento Técnico para funcionamento de Laboratórios Clínicos. http://portal.anvisa.gov.br/documents/10181/2718376/RDC_302_2005_COMP.pdf/7038e853-afae-4729-948b-ef6eb3931b19

6 - Diretrizes para gestão e garantia de qualidade para os Testes Laboratoriais Remotos (Point of Care) – Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial – 2004. http://www.sbpc.org.br/upload/conteudo/320090723141248.pdf

7 - SOCIEDADE BRASILEIRA DE ANÁLISES CLÍNICAS (SBAC) INFORME TÉCNICO DE 25 DE MARÇO DE 2020 - Métodos laboratoriais para diagnóstico da COVID-19. http://www.sbac.org.br/blog/category/coronaviruscovid-19/

8 – Centers for Dieases Control and Prevention (CDC) - Coronavirus Disease 2019 (COVID-19) Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19), Updated April 14, 2020. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

9 – ANVISA - RESOLUÇÃO DE DIRETORIA COLEGIADA - RDC Nº 20, DE 10 DE ABRIL DE 2014 - Dispõe sobre regulamento sanitário para o transporte de material biológico humano. http://portal.anvisa.gov.br/documents/10181/2867956/(1)RDC_20_2014_COMP.pdf/fda4b2b9-fd01-483d-b006-b7ffcaa258ba

10 - D Capitani C, Marocchi A, Tolio A. Automation of the Pre-Analytical Phase: A Performance Evaluation of Alternative Scenarios. Journal of the Association for Laboratory Automation, JALA.2002;7(2).

11- Forsman RW. Why is the laboratory an afterthought for managed care organizations? Clin
Chem. 1996;42(5):813-6.

12 – Wiwanitkit V. Types and frequency of preanalytical mistakes in the first Thai ISO 9002:1994 certified clinical laboratory, a 6 - month monitoring. BMC Clin Pathol. 2001;1:1-5.

13 – Plebani M. Towards quality specifications in extraanalytical phases of laboratory activity. Clin Chem Lab Med. 2004;42(6):576-7.