RECOMMENDATIONS OF THE CLINICAL LABORATORY MEDICAL SOCIETY (SMLC) CHILE FOR LABORATORY DIAGNOSTICS OF SARS-CoV2

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1. BACKGROUND

The Clinical Laboratory is of great support to the diagnosis, evaluation and monitoring of suspected cases of Covid19

The correct use and interpretation of results allows a more accurate and safe clinical management.

The purpose of these recommendations is to provide provisional guidance for practices related to the laboratory diagnosis of SARS-CoV2, including biosafety in the collection, transport, handling, and storage of samples suspected of containing SARS-CoV2, which causes the disease called COVID- 19, by the World Health Organization (WHO). These recommendations will be updated eventually, as new knowledge of the disease emerges.

2.-DEFINITION OF CASES.

According to MINSAL(Chilean Ministry of Health) as of March 18, 2020

2.1.- Suspicious Case

Patient with acute respiratory disease:

• Having fever or at least one sign or symptom of respiratory disease, and with a history of travel or residence in a country / area or territory that reports local transmission of COVID-19 during the 14 days prior to the onset of symptoms.

• You have been in contact with a confirmed or probable case of COVID-19 in the 14 days prior to the onset of symptoms.

• Regardless of the history of travel or contact with a confirmed case of COVID-19 and who presented fever (37.8ºC) and at least one of the following symptoms (odynophagia, cough, myalgia or dyspnea).

• With criteria of severity (presenting fever, cough and respiratory distress) and requiring hospitalization.

2.2-Probable Case:

• Suspicious case in which the laboratory analysis by PCR for COVID-19 was inconclusive.

2.3.- Confirmed Case:

• Suspicious case in which the specific test for COVID-19 was "positive".

At the current epidemiological moment (phase 4) where there is persistent viral circulation in the community and the definition of a suspicious case no longer depends on epidemiological validation, but clinical elements are added, there is the

possibility that any patient who consults with respiratory symptoms is infected with Coronavirus, so biosecurity and PPE measures should be standardized according to this new scenario.

3.- PREANALYTICAL STAGE:

3.1.- Medical Order:
When suspecting a case of COVID-19, the recommended test for the diagnosis of SARS-CoV2 infection is the Polymerase Chain Reaction with reverse transcription in real time (RT-PCR), in upper or lower respiratory clinical samples. It is also recommended to investigate other respiratory pathogens, with the application of molecular panels for other pathogens in the search for the etiological agent.

3.2.-Sampling:
In the case of taking samples with patients suspected of COVID-19, it is recommended to create special identification of these patients, and that they remain for a short time in health centers, waiting for the results of their examinations at home, if their symptoms are not serious, or wait in an isolated area reserved for these patients. Likewise, it is recommended, both for the patient and his companion, the use of a mask while remaining in the unit.

Appropriate respiratory specimens for diagnosis of SARS CoV2 are nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate, alveolar bronchus lavage, bronchial discharge.
The CDC recommends collecting simultaneous samples from the upper respiratory tract (nasopharyngeal and oropharyngeal swabs) for all cases, and from the lower respiratory tract when possible.
In addition to respiratory secretions, the presence of virus RNA has been detected in blood, urine, and stools, but its role in transmission is uncertain and appears to be irrelevant. To date, the presence of SARS-CoV-2 has not been detected in breast milk, therefore it would be suggested to maintain lactation while maintaining all the recommended precautions.

Nasopharyngeal and oropharyngeal swab sampling procedure:

• The person responsible for taking the samples must be a nurse, midwife or

medical technologist, trained laboratory technician
who works in the health center.

• You must gather the material, wash your hands and put on personal protection elements, PPE (N95 mask, procedure gloves, disposable long-sleeved apron or bib, face shield or goggles). Look at annex 1
• Inform the patient about the procedure, it can be with the patient sitting or lying down.

Nasopharyngeal Swab Sample:
• Tilt the patient's head back slightly.
• Insert a flexible swab for the nasopharynx (do not use another type of swab), through the external angle, until reaching the posterior nasopharynx, leaning on the nasal septum.
• Remove visible discharge from the nostrils before starting the procedure
• Gently rotate the swab to obtain a good sample (3 to 5 seconds)
• Gently remove the swab by carefully rotating
• Repeat the procedure with the same swab in the other nostril
• Remove the swab and place it in the MTU medium. Break the swab at the point marked on it. Close the bottle.

Sample Oropharyngeal Swab:
• Place the patient in a comfortable position with good lighting towards the pharyngeal area.
• Ask the patient to pronounce the letter A.
• Scroll the tongue down (with low tongue).
• Insert the rigid swab until reaching the oropharynx, rub gently taking care not to touch the tongue. Do not use cotton swabs, alginate or a wooden stick.
• Remove the swab and introduce it in the MTU medium (or in sterile PBS).
• Cut the swab in a way that allows the tube to be closed
• Leave the swab in the middle and close the tube tightly
• Label the sample and record the procedure
• Dispose of all disposable supplies in yellow containers with a yellow bag (REAS standard).
• Withdraw the PPE (See annex 2)
• Handwashing.
• Send to the laboratory.

3.3.-Transport of samples:
• The forms or medical order must come separate from the sample.
• The official who will transport the samples must be informed that there are samples from a suspected case of Coronavirus
• The transport of samples that may contain 2019-nCoV must use a triple packaging and comply with international standards related to the transport of infectious substances: "Biological substance, category B".

• It is not recommended to use pneumatic transport systems due to the risk of aerosol release
• Respiratory samples must be transported in a viral transport medium (MTU) or, alternatively, in PBS, in a refrigerated condition at 4ºC since it is an RNA virus and thus the risk of deterioration of the sample is avoided.

4.- ANALYTICAL STAGE:
4.1.- Handling of samples within the laboratory: According to CDC recommendations, any procedure that potentially generates aerosols (eg aliquot, vortex, or centrifuge open tubes) should be handled in a Biosafety cabinet. Procedures that generate aerosols and are performed outside the biosafety cabinet, as well as cleaning highly suspicious clinical material, must be done with the use of the N95 mask or equivalent, gloves, bibs and goggles. After samples have been processed, surfaces and equipment must be decontaminated with appropriate disinfectants.
4.1.1.-The following procedures can be performed at BSL-2 Laboratory facilities using standard work practices:
• Plate configuration for molecular analysis of viral nucleic acids already extracted.
• Routine (visual) examination of bacterial and fungal cultures.
• Routine staining and microscopic analysis of fixed smears.
• Final packaging of samples for transportation to diagnostic laboratories for additional testing.
• Inactivated samples (samples in nucleic acid extraction buffer)
• Serum, plasma or whole blood samples for measurement of biochemical, hematological, hormonal tests, etc., which are introduced covered to the automated equipment.
4.1.2.- The following procedures must be performed in a Class II Biosafety Cabinet: • Aliquot and / or dilute samples
• Inoculation and seeding of bacterial or mycological culture media.
• Nucleic acid extraction procedures with potentially infected samples. Preparation and chemical or thermal fixation of smears for microscopic analysis

4.2.-SARS CoV2 identification

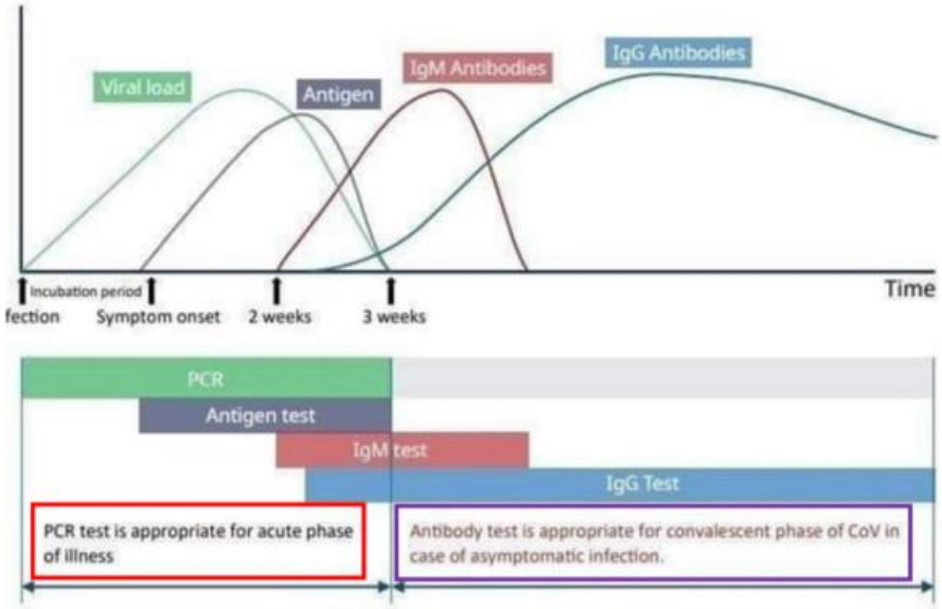
The WHO has recommended carrying out mass testing, since the strategies that combine early confinement and mass testing are those that have been shown to obtain better results in reducing the mortality of the disease. However, there are financial resource limitations, low reagent availability, and low availability of trained personnel to perform the tests. Confirmation of cases and their quarantine is a measure with a significant impact on flattening the curve of infected cases.
4.2.1.- RT-PCR: RT-PCR is the test with the highest sensitivity and considered confirmatory of the diagnosis of COVID-19. Viral targets that are amplified include

the N, E, S, and RdRP genes (at least two of them). Nucleic acid detection sensitivity is variable and taking the right sample at the right time of disease is key.

4.2.2.- Antigen Detection: Antigen detection has low evidence, due to its recent introduction and lack of experience of use. Even so, it is a rapid test (results in less than 30 min, vs RT-PCR that takes about 4 hrs), it has a good positive predictive value, therefore it should also be considered for diagnosis. In correlation with RT-PCR, studies have shown that it has a higher sensitivity the more copies of the virus there are in it.

4.2.3.- Detection of IgM / IgG Antibodies:

Antibody detection takes longer to appear than antigen or nucleic acid detection. Studies have been reported where increases in antibodies were observed from 10 days after the onset of symptoms, first IgM and then IgG. It is a quick test (takes less than 30 min) and has a good PPV. The sensitivity of this type of test can be improved when used as a serological follow-up for 2 to 4 weeks. Before performing any of these diagnostic tests, it is of great importance to know the onset of symptoms (Figure 1), since the PCR becomes negative from day 8 onwards, from the onset of symptoms in nasopharyngeal samples and thereafter and up to maximum on day 22 it would only be positive in sputum (lower respiratory tract) or stool samples. As of day 7 of the onset of symptoms, it is recommended to use an IgM / IgG Antibody detection test as a complement, being as of the 7th day positive in 50% of patients, on the 10th day in 70% and in the 14th day from the onset of symptoms in 100% of patients. Therefore, the IgM / IgG Antibody test should be considered in patients who present with advanced disease with more than 7 days of symptoms, patients with negative PCR and symptoms, epidemiological studies, contacts and healthcare personnel.



COVID-19

Figure1: SARS CoV2 specific laboratory

4.3.- Other Laboratory studies in SARS CoV2 + Patients:
Hospitalized patients with COVID19 +, should be evaluated systemically. This is why we propose the following laboratory tests as a “COVID-19 profile”, to manage and establish the prognosis of hospitalized cases.

• Blood count:
The white blood cell count is generally normal, although it may be increased or decreased.
Lymphopenia is common and occurs in ~ 80% of patients (Guan et al, Yang et al).
Mild thrombocytopenia is common (but platelet counts rarely drop below 100,000).
Lower platelet counts are a sign of poor prognosis (Ruan et al 3/3).

• Coagulation tests:
Elevated levels of D-dimer are common in this disease. The condition may evolve with intravascular coagulation disseminated over time, which is related to a worse prognosis (Tang et al. 2020).

• Inflammatory parameters:
Procalcitonin: In patients hospitalized for COVID-19 infection, an elevation of procalcitonin could indicate a bacterial superinfection or poor prognosis.
C-reactive protein (PCR): COVID-19 infection increases PCR. This elevation seems to be related to the severity of the condition and poor prognosis.
VHS: Increased
IL-6: Increased
Ferritin: If it is increased, it is associated with a poor prognosis.

• Liver Profile:
Albumin: Decreased
GOT: Increased
GPT: Increased
Bilirubin: Increased

• Gasometry:
pH: Decreased (acidosis)
pO2: Decreased

Increased pCO2
Lactate increased in patient in Shock
PaO2 / FiO2: Low 300 in severe cases

• Cardiac Markers:
LDH: increased
CK: increased total
Increased troponin, associated with myocardial damage, is associated with a poor prognosis.

• Kidney Profile:
Creatinine: increased
Urea / BUN: Increased, associated with poor prognosis

5.- Post Analytical STAGE:

5.1.-Validation and confirmation of SARS CoV2
Laboratories that are certified by the ISP (Public Institute of Health) to make the direct report of SARS CoV2 results can issue their results directly, without sending the sample for confirmation. Laboratories that are not certified by the ISP to carry out a direct SARS CoV2 report, must send the sample for confirmation to a laboratory that is certified, using triple packaging and complying with international standards related to the transport of infectious substances: " Biological substance, category B ", with the separate forms of the samples.

Regarding the centrifugation of samples, it is generally recommended that a safety cap be used on the centrifuges and wait until the generation of aerosols is safe before opening. A N95 mask should be used if there is a risk of aerosols or the process must be carried out in a class II biosafety cabinet.

Appendix 1

Steps to put on personal protective equipment (PPE) including gown

1 Remove all personal items (jewelry, watches, cell phones, pens, etc.)

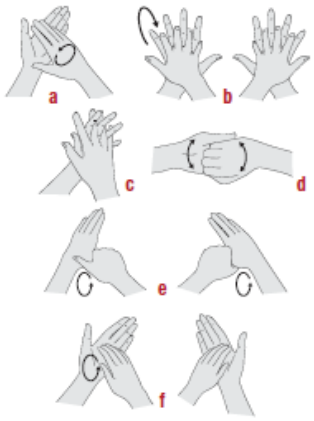
2 Put on scrub suit and rubber boots[1] in the changing room.

3 Move to the clean area at the entrance of the isolation unit.

4 By visual inspection, ensure that all sizes of the PPE set are correct and the quality is appropriate.

5 Undertake the procedure of putting on PPE under the guidance and supervision of a trained observer (colleague).

6 Perform hand hygiene.

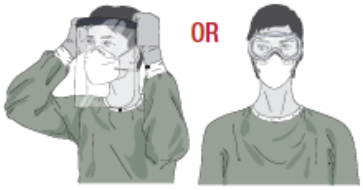


7 Put on gloves (examination, nitrile gloves).

8 Put on disposable gown made of fabric that is tested for resistance to penetration by blood or body fluids OR to blood-borne pathogens.

9 Put on face mask.

10 Put on face shield OR goggles.



11 Put on head and neck covering surgical bonnet covering neck and sides of the head (preferable with face shield) OR hood.

12 Put on disposable waterproof apron (if not available, use heavy duty, reusable waterproof apron).

13 Put on second pair of (preferably long cuff) gloves over the cuff.

[1] If boots are not available, use closed shoes (slip-ons without shoelaces and fully covering the dorsum of the foot and ankles) and shoe covers (nonslip and preferably impermeable)

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Appendix2

Steps to take off personal protective equipment (PPE) including gown

1 Always remove PPE under the guidance and supervision of a trained observer (colleague). Ensure that infectious waste containers are available in the doffing area for safe disposal of PPE. Separate containers should be available for reusable items.

2 Perform hand hygiene on gloved hands.[1]

3 Remove apron leaning forward and taking care to avoid contaminating your hands. When removing disposable apron, tear it off at the neck and roll it down without touching the front area. Then untie the back and roll the apron forward.

4 Perform hand hygiene on gloved hands.

5 Remove outer pair of gloves and dispose of them safely.
Use the technique shown in Step 17

6 Perform hand hygiene on gloved hands.

7 Remove head and neck covering taking care to avoid contaminating your face by starting from the bottom of the hood in the back and rolling from back to front and from inside to outside, and dispose of it safely.

OR

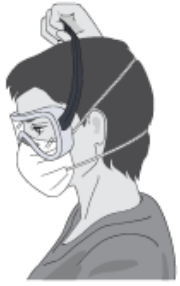
8 Perform hand hygiene on gloved hands.

9 Remove the gown by untying the knot first, then pulling from back to front rolling it from inside to outside and dispose of it safely.

10 Perform hand hygiene on gloved hands.

11 Remove eye protection by pulling the string from behind the head and dispose of it safely.

OR



12 Perform hand hygiene on gloved hands.

13 Remove the mask from behind the head by first untying the bottom string above the head and leaving it hanging in front; and then the top string next from behind head and dispose of it safely.

14 Perform hand hygiene on gloved hands.

15 Remove rubber boots without touching them (or overshoes if wearing shoes). If the same boots are to be used outside of the high-risk zone, keep them on but clean and decontaminate appropriately before leaving the doffing area.[2]

16 Perform hand hygiene on gloved hands.

17 Remove gloves carefully with appropriate technique and dispose of them safely.

18 Perform hand hygiene.

1 While working in the patient care area, outer gloves should be changed between patients and prior to exiting (change after seeing the last patient)

2 Appropriate decontamination of boots includes stepping into a footbath with 0.5% chlorine solution (and removing dirt with toilet brush if heavily soiled with mud and/or organic materials) and then wiping all sides with 0.5% chlorine solution. At least once a day boots should be disinfected by soaking in a 0.5% chlorine solution for 30 min, then rinsed and dried.

World Health Organization

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