PROTOCOL OF EVALUATION OF DIAGNOSTIC TESTS FOR SARS-CoV-2

1. Introduction

This protocol is a joint action among the Brazilian Society of Clinical Pathology / Laboratory Medicine (SBPC/ML), Brazilian Society of Clinical Analysis (SBAC), Brazilian Association of Diagnostic Medicine (ABRAMED) and the Brazilian Chamber of In Vitro Diagnostics (CBDL), support from the International Diagnostic Centre (IDC) of London School of Hygiene & Tropical Medicine (LSHTM) and Latin American Alliance for the Development of In Vitro Diagnostics (ALADDIV) in order to deal with the COVID-19 epidemic, which started in Wuhan, China, and that has resulted in the current global pandemic we are facing.

1.1 Objectives:

1.1.1. Evaluation of Diagnostic Kits for SARS-CoV-2 available in the Brazilian market;

1.1.2. Share and publish the results of such evaluations on a frequent basis in order to serve as a reference for the public and private sectors;

1.1.3. Generate data for future publications about regulatory convergence, access, "preparedness" for future epidemics, among other topics.

1.2 Minimum requisites for the participating laboratories

The participating laboratories should agree and comply with the following requisites:

a- To have direct access to the collected material and the capacity of keeping it in the adequate conditions

b- To have its own laboratory, including at least one located inside a hospital infrastructure with COVID-19 patients;

c- To count in its clinical team highly experienced professionals with master or phD degrees.

d- To count on a system that allows the follow up and tracking of patients from the clinical and laboratory point of view.

2. **Scope:**

Study of validation for laboratory tests related to the diagnostic, follow up and prognosis of SARS-CoV-2 and COVID-19. It is foreseen the validations of:

1. **REMOTE LABORATORY TESTS (IMMUNOCROMATOGRAPHY)** or **SEROLOGICAL AUTOMATED TESTS** for identification of total IgG and IgG and IgM antibodies.

2. **REMOTE LABORATORY TESTS (IMMUNOCROMATOGRAPHY)** or **SEROLOGICAL AUTOMATED TESTS** for identification **of** antigens.

3. **REMOTE LABORATORY TESTS OR POCT'S** for identification of SARS-CoV-2 by different technologies.

4. **LABORATORY TESTS BY DIFFERENT METHODOLOGIES** - CLIA, CMIA, PCR, etc.

3. Sample Types:

In general, there are the following possibilities:

Capillary blood, serum or plasma – the recommended anticoagulant is the one indicated by the manufacturer (EDTA, heparine or sodium citrate).

The sample should be separated in serum/plasma as soon as possible in order to avoid hemolysis.

Samples of nasopharynx material, bronchial secretion and bronchoalveolar lavage should be collected in a swab supplied by the manufacturer or another way, as per the orientation of the manufacturer. The collected samples should be put in the proper container, transported and stored as per indications of the package insert.

The collected materials that are diverse from the above mentioned (urine, stool, etc) should be treated in accordance to specification.

4. Samples stability:

In general:

The test should be performed within 8 hours after the sample collection.

Do not leave the sample in room temperature for too long.

The samples can generally be stored from 2-8°C for periods up to 3 days.

During long storage periods, the samples should be kept below -20 ° C for up to 9 days or at – 70% for up to 6 months.

We remind that the stability may vary in accordance with the manufacturer's instruction. So, it should be described in this item what is the stability in accordance with the manufacturer.

5. Studies to be performed:

The following studies are supposed to be performed with the new diagnostic kits for COVID-19.

5.1 Initial assumptions:

a. **Negative samples:** to consider samples, preferably collected until December 2019 (when coronavirus was not yet found in Brazil). Preferably samples known to be negative for HIV, hepatitis B and C.

   Complementary samples should be included for testing cross reaction with positive samples for Mononucleosis and low avidity, high and low atypia, positive samples for Dengue and Herpes.

b. **Positive samples:** consider samples from patients with diagnosis of COVID-19 by RT-PCR, collected in the following time intervals from the symptom beginning:

   Between 7 and 14 days

   Between 15 and 21 days

   After 21 days.

c. Important to register the time of the initial moment of the symptoms (or hospitalization) and time between the first diagnostic by RT-PCR and the collection for the rapid test.

d. The serological kits, CMIA, ELISA, etc. should only be used after the realization of the controls recommended by the manufacturer in each routine..

e. In order to assure reliability of the rapid test custody chain, for each lot / shipment should be done a positive and negative control and also at all times that new battery of tests of patients' samples should be ran.

f. It is recommended that for each period indicated in item 5.1.b, at least 20 samples should be evaluated.

g. The cut-off values to be adopted should necessarily be the ones indicated by the manufacturer in the kit package insert.

5.2 Performance Study:

The following data should be calculated using the attached spreadsheet:

a. Sensitivity: reactives in the assay /total of confirmed positives (RT-PCR)
b. Specificity: total of non-reactives in the assay / total of confirmed negatives
c. PPV (positive predictive value): true positives / total positives
d. NPV (negative predictive value): true negatives / total negatives
e. Accuracy: (true positives + true negatives) / total samples
f. PLR (positive likelihood ratio): sensitivity / (1 – specificity)
g. NLR (negative likelihood ration): (1-sensitivity) / specificity
h. DLR (diagnostic likelihood ratio): PLR/NLR

The items a to c are expressed in percentage

5.3 Imprecision Study:

This study will be performed only for **serum or plasma** (see study at the manufacturer package insert). Although it may be performed with control samples, it is recommended, preferably, to perform with patients' samples.

4.2.1 Repeatibility (intra-assay): analyse 12 replicates of three samples, being one high positive, one low positive and one negative, preferably with three different lots of the kit.

4.2.2 Intralaboratorial (inter-assay): analyse 12 replicates of three samples, being one high positive, one low positive and one negative, in different days, what can be done in 4 dosages x 3 days.

It should be done 20 replicates, 4 dosages in 3 days with the aim of publication in the shortest possible time, considering that all the assays should be ran simultaneously in the same samples

5.4 Correlation study:

Although the RT-PCR is the method of choice, it is known that there is an immunological window for the seroconversion, even though it is not yet well known for COVID-19.

5.4.1. Samples

Realization of, at least, 100 samples, being at least 50% of these samples confirmed as being positives (see item 5.1b), 40% of negative samples and 5 to 10% of samples close to the detection limit or cut off.

Suggestion: To use 60 samples with previously known results:

· 40 samples IgM

- 10 high positives

- 10 true negatives

- 7 low viral load positives

- 7 medium viral load positives

- 6 low viral load positive

· 20 samples IgG

- 5 positives with negative IgM

- 5 negatives with negative IgM (without contact)

- 3 positives IgG with positive IgM, low viral load

- 4 positive IgG with positive IgM, medium viral load

- 3 positive IgG with positive IgM, high viral load

The serial sample collection should be done in the intervals indicated in item 5.1.b.

The kits that recommend the use of capillary sample, besides the serum or plasma samples, collect 01 capillary sample and do the test by the bedside and register the result and collect also a serum sample and another with anticoagulant (recommended by manufacturer)

Perform the collection in accordance with the manufacturer's instructions.

Send the whole blood samples (serum or plasma) to the laboratory for its preparation and performance of the test.

Register in a spreadsheet all the data, using the attached model.

5.4.2 Technical procedure

Realize the procedure in accordance to the manufacturer's orientation.

The procedure with the capillary sample, as well as the realization of the procedure in serum or plasma should only be done by trained professional.

6. Caractheristics of expected performance:

We expect results with equivalent performance to the one found in the manufacturer's study, as indicated in the kit package insert.

However, the kits should be evaluated in relation to its performance in a wide spectrum, orienting indications and limitations in what its use is regarded.

7. Approval:

Approval should be done in accordance with the results found and the objectives of use of the kit and its adequacy to the immunological window.

The final evaluation should also bring the limitations of use (such as immunological window, epidemiological data, etc); warnings (examples: in relation to quality control or reproducibility); and other appropriate limitations.

More information: www.testecovid19.org