COVID-19 PANDEMIC

TESTING FOR COVID-19: WHEN, WHO, AND WHAT TEST?

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ABSTRACT • The laboratory diagnosis of COVID-19 is essentially based on three modalities. The reference method in lieu of viral culture is the molecular amplification assays, mostly by using the real-time reverse-transcriptase polymerase chain reaction (RT-PCR). However, due to its complexity, needed equipment and expertise as well as the high expense, other assay modalities have been introduced. These include antigen-based and antibody-based detection assays. This article provides a consolidated approach that will shed light on the different formats and utilities that these assays are intended for at this stage in time, addressing when, who, and what in the testing of COVID-19. It is worth noting that a combination of different assays is advisable to settle interpretation of test results to reflect the true scope of the patient's condition.

Keywords : COVID-19; lab diagnosis; rapid testing

INTRODUCTION

The laboratory diagnosis of COVID-19 has been rapidly and dynamically evolving [1-6]. Three types of laboratory diagnostic assays have been used in the diagnosis and evaluation of exposure to COVID-19, namely molecular, antibody and antigen detection tests. The RT-PCR, performed on respiratory specimens, is the reference standard for COVID-19 diagnostics [4-6].

Currently, very few tests for COVID-19 investigation have been approved by the US Food and Drug Administration (FDA). This is done in order to facilitate their use by the clinical laboratories aiming at expanding the testing towards assisting in controlling virus spread. Many molecular and serologic immunoassay technologies have received Emergency Use Authorization (EUA) from the FDA for both hospital laboratory-developed assays and for several commercial kits [4-6].

Developed and employed tests/assays should be validated before use. Despite the variation in sensitivity, specificity and objective of utilization, the developed tests have been anticipated to provide reliable and rapid turnaround time (TAT) results in identifying infected or exposed cases. These assays are essential to control the viral spread and transmission, to initiate appropriate protective measures, and to start treatment when applicable. In addition, these assays can be used in epidemiologic surveillance and forecasting exposure to the community, helping the health policy makers initiate appropriate control measures and actions.

Overall, a successful laboratory diagnosis necessitates keeping in mind two important key factors: the correlation of test results with clinical picture/history and the need to use a combination of two testing modalities. This is necessary for appropriate interpretation of COVID-19 case definition/status as being proven, suspected, or asymptomatic/exposed, as well as any other relevant decision a test result will help to inform [4-6].

The text that follows addresses the three main laboratory diagnostic modalities for COVID-19 case diagnosis or exposure:

- Molecular tests, also known as Nucleic Acid Amplification Tests (NAAT) that target detection of specific nucleic acid sequences of the virus;
- Antigen detection tests: detection of viral antigenic epitopes;
- Antibodies detection tests: detect the humoral immune response (e.g. IgM, IgG) following an infection.

MOLECULAR LABORATORY DIAGNOSIS OF COVID-19

Introduction

Molecular assays are considered the cornerstone and reference method for the lab diagnosis of the COVID-19 infection [4-6]. The discussion that follows will address the viral aspects as it relates to the molecular diagnostic methods, emphasizing the RT-PCR and its utilization in the diagnosis of viral diseases.

Description and classification of the molecular lineages of human coronaviruses were first identified in the mid-1960s. This warranted the understanding of current

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molecular tests used for the diagnosis of this virus. Four main sub-groupings of coronaviruses were revealed as: alpha, beta, gamma, and delta [7; Refer to the virology article in this special issue by Bizri AR].

Few are known to cause infection among humans, a couple belong to the alpha coronaviruses (229E, NL63), while the rest are Beta-CoVs. The greatest clinical importance concerning humans of the Beta-CoVs are OC43 (which can cause the common cold) and HKU1 of the A lineage, SARS-CoV and SARS-CoV-2 (which causes the disease COVID-19) of the B lineage and MERS-CoV of the C lineage [8].

As the clinical description of COVID-19 continues to evolve and transmission of the disease by asymptomatic individuals progresses, widespread testing has become a necessity, warranting the probing in molecular testing [9-10].

Viral genome sequence events of COVID-19

On January 4th, 2020, the Food and Drug Administration (FDA) issued emergency use authorization (EUA) to enable Centers for Disease Control and Prevention (CDC) to offer molecular diagnostic tests for COVID-19. The complete viral genome sequence was released for immediate public health support via the community online resource virological.org on January 10th, 2020, (Wuhan-Hu-1, GenBank accession number MN908947), followed by four other genomes deposited on 12th January in the viral sequence database curated by the Global Initiative on Sharing All Influenza Data (GISAID). On January 17th CDC developed and validated the first molecular test for COVID-19 detection and on January 24th, CDC publicly posted the assay protocol for this test. All along, close cooperation and continuous monitoring to evolve such diagnostic tools have been ongoing among CDC, FDA, World Health Organization (WHO) and advisories from other reference labs worldwide for updates.

Type/methods of molecular assays

Rapid evolution in molecular assays has been ongoing. For example, the earlier hybridization methods, which were used for identification of pathogens were not sensitive enough for their detection. They were superseded by NAAT, which makes millions of copies of a specific section of the pathogen genome, amplifying small amounts to detectable levels.

Several amplification methods have been developed for the identification of COVID-19 including RT-PCR, nucleic acid sequence based amplification (NASBA), transcription mediated amplification (TMA), strand displacement amplification (SDA) and loop mediated isothermal amplification (LAMP) [11].

RT-PCR-reference method for COVID-19 laboratory diagnosis

In COVID-19 exposure or infection, RT-PCR is globally known as the mainstay and reference method for its laboratory diagnosis. It is highly sensitive and specific in its ability to detect the virus in people suspected of being exposed (asymptomatic) or to confirm its diagnosis in symptomatic patients. [12].

Gene targets for RT-PCR and other NAAT molecular diagnosis

WHO and the European Center for Disease Prevention and Control (ECDC) via GISAID have published the different SARS-CoV-2 specific target genes (*E*-gene, *ORF1* and *N*-gene), genome positions, amplicon length, institutes along with the corresponding available protocols. Details of these are noted in the link together with the rate of mutation in the listed primers and probes. [https://www.who.int/who-documents-detail/molecular-assays-to-diagnose-covid-19-summary-table-of-available-protocols] [https://primerscan.ecdc.europa.eu/?assay=Overview, 4/28/2020, www.eurosurveillance.org].

These protocols are shown in the Table and they are under continuous revision and update by Foundation for Innovative New Diagnostics (FIND) and WHO. [https://www.finddx.org/covid-19/]; [file:///G:/COVID-19/FIND COVID-19-GUIDE_24.03.2020.pdf. https://www.finddx.org/covid-19/pipeline/ For an overview of COVID-19 diagnostics that are currently available or in development. https://www.360dx.com/coronavirus-test-tracker-launched-covid-19-tests: Coronavirus Test Tracker: Commercially Available COVID-19 Diagnostic Tests].

TABLE

SUMMARY TABLE OF AVAILABLE PROTOCOLS IN THIS DOCUMENT

Institute Gene	Targets
China CDC, China	*ORF*1ab and *N*
Institut Pasteur, Paris, France	Two targets in *RdRP*
US CDC, USA	Three targets in *N* gene
National Institute of Infectious	Pancorona & multiple targets
Diseases, Japan	Spike protein
Charité, Germany	*RdRP, E, N*
HKU, Hong Kong SAR	*ORF*1b-*nsp*14, *N*
National Institute of Health, Thailand	*N*

Commercial molecular platforms

Currently, the performance characteristics of COVID-19 PCR testing available in the market are not well established as clinical trials were not performed prior to the reagents being released under a EUA status by the FDA. These were originally released under Research Use Only (RUO) while waiting for EUA approval. The slow im-

plementation of testing and a lack of testing capacity are due to a lack of: positive control materials, personnel/time, primers/probes, specificity panel, funds, quality control system, commercial tests, procurement procedures, training and equipment. However, every laboratory should perform test validation on each EUA granted test kit before use through implementation of quality control (QC) and in accuracy and precision studies [13]. Examples of available RT-PCR and other molecular platforms include: Applied Biosystems® 7500, BioRad CFX96TM, Cepheid SmartCycler®, Cobas® Z480, LightCycler® 2.0, Rotor-Gene® 6000, Abbott Molecular, Becton Dickinson BD MAX™ System, SeeGene. These have TAT that vary from around more than one hour up to 150 minutes.

However, rapid TAT and performance as point of care, as an estimate of less than one hour, were reported for Roche ID NOW COVID-19 (5 minutes), Qiagen QiaStat-Dx (43 minutes) and Cepheid Xpert® Xpress SARS-CoV-2 (45 minutes).

Diagnostic kits

Updates on FDA/WHO approved *in-vitro* diagnostic kits having EUAs/CE/RUO for COVID-19 testing on the various platforms based on the different target genes available globally, including Lebanon, are (alphabetically):

- Abbott RealTime SARS-CoV-2 Assay, (RdRp and N genes), (Abbott Molecular);
- AccuPower® COVID-19 Real-Time RT-PCR Kit, (E gene and RdRp gene), Bioneer;
- Allplex™ 2019-nCoV Assay, (E gene, RdRP gene, N gene), (SeeGene);
- BioGX SARS-CoV-2 Reagents, (N1, N2 and RP gene), (Becton Dickinson);
- CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, (N1, N2 and RP gene), (CDC);
- Cepheid Xpert Xpress SARS-CoV-2 assay, (N2/E gene), GeneXpert Infinity Systems;
- Cobas SARS-CoV-2, (ORF-1a/b and pan E-gene), (Roche Molecular Systems, Inc.);
- ID NOW COVID-19 assays, (RdRP), (Abbott Molecular);
- Panther Fusion SARS-CoV-2, (ORF 1ab (ORF1a/ORF1b gene), (Hologic, Inc);
- PerfeCTa SYBR Green FastMix, (open system), Quantabio;
- QIAstat-Dx Respiratory SARS-Cov-2 assay, (Orf1b poly gene (Rdrp gene) and E genes), (Thermo Fisher Scientific, Inc);
- RealStar® SARS-CoV-2 RT-PCR Kit RUO, (E and S gene), Altona;
- SARS-COV-2 R-GENE, (N, RdRp andE gene), (bioMérieux SA);
- Script® One-Step qRT-PCR Kit, (open system), Invitrogen;
- TaqPath COVID-19 Combo Kit, (ORF1ab, N Protein and S Protein gene), (Thermo Fisher Scientific, Inc.).

These kits have high sensitivity and specificity, and their TAT for test performance varies from around 5 to 150 min. [www.fda.gov › media] [https://www.fda.gov/medical-devices/emergency-situations medical-devices/emergency-use-authorizations.]; [file:///G:/COVID-19/FIND_COVID-19-GUIDE_24.03.2020.pdf. https://www.finddx.org/covid-19/pipeline/ For an overview of COVID-19 diagnostics that are currently available or in development.]

Specimen collection and management

The specimens, mainly respiratory, are obtained from patients who fulfill a case definition of COVID-19, while using appropriate personal protective equipment (PPE) and testing under biological safety laboratory level-2 (BSL2) [14]. Failure to abide by good laboratory practices will lead to lab contamination, risk of infection and invalid test results. The collected specimens are placed into virus transport medium (VTM) and forwarded immediately to the lab for molecular testing. In case of delay in testing, specimens need to be appropriately stored, while maintaining stable cyclic threshold (Ct) values, as follows: –4°C for up to 72 hrs, and –70°C for a longer period. The extracted RNA can be stored at –70°C or lower for long periods. In the shortage of VTM swabs, regular flocked/polyester swabs in a normal saline/phosphate buffer solution/tissue culture solution are all acceptable. [https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html].

Heat inactivation of samples (56°C for 30 minutes) after collection from patients and before performing PCR are necessary, in order to avoid infection and health hazards during transport and in the preanalytical and analytical phases [15].

The quality of results depends significantly on the quality of sample collection, the length of time the patient has been symptomatic, and the viral burden at the time of testing. The sensitivity of PCR-based testing is generally very high, when a good sample is obtained.

Specimen types and PCR detection rates

The respiratory specimens are the main test for detecting or confirming COVID-19 infection, though other specimen types were also investigated. For example, a study conducted by Wang et al., revealed the detection rates in different specimens to be: 93% in bronchoalveolar lavage (BAL), 72% in sputum, 63% in nasal swab, 46% in fibrobronchoscope brush, 32% in pharyngeal swab, 29% in feces, 1% in blood and 0% in urine [16]. Lately, saliva

samples (a noninvasive alternative) revealed importance as a source of molecular virus recovery and IgA detection. This is attributed to the fact that saliva has the highest viral load near presentation, and can account for the fast-spreading nature of this epidemic [17]. However, simultaneous collection from multiple respiratory sites (combined in one vial) for testing is advisable in order to improve the sensitivity and reduce false-negative PCR test results.

Duration of viral detectability

Studies attempted to answer the question on when can the virus be detected before symptoms onset and for how long thereafter.

Arons et al., using cell culture, reported that viable virus was recovered 6 days before to 9 days after the first evidence of symptoms [18]. Wolfel et al. 2020, reported that the virus was isolated during the first week of symptoms from 16.66% in throat swabs, and 83.33% in sputum samples. No isolates were recovered from samples taken after day 8. The virus isolation success depended on viral load: samples containing < 100,000 copies/mL (or copies per sample) never yielded an isolate. They also suggested that such a load was unlikely to be infectious [19]. Gautret et al. reported in an observational study of 80 inpatients treated for 3 days that the PCR test of nasopharyngeal viral load rapid fall: 83% negative at Day 7, and 93% at Day 8 [20]. Zou et al. also indicated that COVID-19 viral load from nasal and throat swabs decreased close to detection limits 12 days after onset of symptoms, and from saliva in 11 days [21].

Details for timings of the specimen's collection from symptomatic patients and contacts and for each transmission scenario are clearly stated in both WHO guideline interims. Patients' viral loads in the nasopharynx is highest around the time of symptom onset. [22]. Kim et al. conducted a follow-up study on the kinetics of viral load in quarantined patients infected with COVID-19 during the first 14 days of exposure. The asymptomatic patients showed Ct values > 35 while the presymptomatic (had highest viral load) showed Ct values < 20. Live virus couldn't be recovered from culture in PCR of Ct > 35 [23].

Persistence of positive PCR after resolution of infection

The aforementioned studies and others indicate that detection of active virus by PCR post 14 days of infection (post onset of clinical signs) in an individual who is symptom free and no longer infectious is unlikely [18-21]. However, the persistence of molecular (PCR) positive test results lingering for several weeks (up to 8 weeks if not more) despite patients/individuals being recovered and becoming asymptomatic has been encountered [24].

This can constitute a challenging global dilemma for physicians, laboratory directors, and for health authorities, especially in the lack of access to COVID-19 cell culture that can determine for these unique patients if they do have functioning infectious viral particles or just residual RNA. Again, and based on the aforementioned studies, one can most likely interpret that the PCR in this situation is picking up specific segments of the nucleic acid/residual nucleic acid of an inactive virus and cannot be equated to an infectious organism. In this situation, resorting to other tests such as the rapid antibodies and/or antigen detection tests would be warranted in helping resolve this problem taking into consideration the overall clinical history and current situation of the individual.

Testing approach and algorithm

Testing for COVID-19 virus is a two-step process, involving first a screening assay for sarbecovirus Subgenus (both SARS virus and COVID-19 virus), and if positive followed by a confirmatory assay for COVID-19 virus only.

The best time to test a COVID patient using a molecular assay is early in the course of disease. In symptomatic COVID patients, SARS-CoV-2 viral RNA can be detected about 1 day prior to symptom onset and remains detectable at high levels for about 6-7 days. Then it substantially decreases to negligible levels after 10 days post symptom onset, and typically does not represent infectious virus, though PCR can remain positive for sometime due to its high sensitivity in detection of nonviable genetic particles of a dead virus. Retesting is advised for initially negative PCR patients with a deteriorating respiratory clinical course consistent with COVID-19 infection and have had exposure to a COVID-19 positive individual [22]. Suspected patients with repeated negative PCR testing, up to 3 times at least 24hrs apart in an upper respiratory specimen, should be tested with an alternative specimen type. Differences on viral loads between specimen sources and the sensitivity of the assays were detected in multiple studies [16,21].

Interpretation of results

The differences in the performance of the molecular test protocols are affected greatly by the mismatches that are likely to arise from primer design rather than by virus mutation and these are mainly Charite Germany *RdRP* and Japan NIID *N*-gene [https://primerscan.ecdc.europa.eu/?assay=Overview,4/28/2020, www.eurosurveillance.org].

Combination of PCR results in both screening and confirmatory genes in different protocols have led to increased detection and specificity [25-27].

A load result between 0 and 40 can be obtained, and this is called the Ct value or threshold cycle. If a sample

has Ct of over 35, the viral load is low. The level of Ct correlates to disease severity. According to the WHO accepted protocols there is no clear standardized Ct threshold used and there should be two positive genes in order to report samples as COVID-19 positive. In one study, Leiberman et al. assessed the performance characteristics of five separate molecular assays for the detection of SARS-CoV-2. They showed 100% sensitivity and specificity for all samples with high viral load (Ct < 35). Inconclusive/discordant specimens had low viral titers (Ct > 37). A combination of the two genes resulted in better detection of the positive samples especially of high Ct [12].

A recent multicenter study that compared the Ct values in relation to different target genes, showed that E target had lower Ct values than the *N2* and *RdRp* targets, while the *RdRp* target was consistently the least sensitive with NPA of 74%. The combination of *E* and *N2* targets provided the highest sensitivity across the range of specimen types tested, and therefore the *RdRp* target was excluded in the EUA version of the test [28].

Testing at Rafik Hariri University Hospital (RHUH)
At RHUH, the main governmental CoVID-19 designated center in Lebanon, COVID-19 testing is performed using primers sequences and probes separately of the COVID-19 virus. These sequences are directly purchased from TIBMol BIOL or through commercial companies such as Roche (LightMix® - Roche Diagnostics) adapted for the Charité Germany protocol. [https://www.who.int/who-documents-detail/molecular-assays-to-diagnose-covid-19-summary-table-of-available-protocols ; ref-005-procurement-lab-request-list.pdf, www.who.int › ref-005-procurement-lab-request-list].

Around 20,000 tests were performed as of 27-5-2020. False negative and false positive results, in this test and others, can be expected during high or low viral prevalence, respectively. However, to ensure reliability of testing, the WHO recommendation in random confirmation of 30% among positive specimens is followed. Also, all positives for *E*- gene are repeated twice with a confirmatory test using the German protocol that targets *RdRP* gene. Positive results are reported based on the WHO recommendations if both the *E* and *RdRP* genes reveal positive results. E-gene results of Ct > 35 rarely displayed positive results for the *RdRP* confirmatory gene. Concerning clinical classification of cases based on Ct values, this aspect remains pending.

In conclusion on molecular testing
Molecular assays, to-date, are the standard reference tools in use to diagnose and confirm COVID-19 infection in symptomatic or asymptomatic individuals. However, their limitations include: requirement of sophisticated equipment, need of special training, complicated assay procedures and use of dedicated reagents. Such complex requirements prompted the search for other diagnostic modalities such as antigen-based and antibody-based detection

ANTIGEN DETECTION-BASED DIAGNOSIS

Background-relevance
Antigen detection-based diagnosis of infectious etiologies utilizes different assays to detect and identify the etiologic agent. Like molecular assays which target detection of specific nucleic acid sequences compatible with the virus in the respiratory specimens, antigen detection assays are another approach used to detect parts (antigenic epitopes) of the novel COVID-19 virus as an indicator/marker of the viral infection in the same type of specimens. They are similar to PCR in utilization purposes of detecting new cases. However, compared to molecular assays, antigen detection assays don't need expensive machines, faster in TAT (provide yes-or-no-results on the spot), cheaper and easier to perform, but their sensitivity and specificity still need fine tuning to match that of the PCR [4-6,29].

Antigen-based detection tests and formats
To date, a couple of in-vitro diagnostic kits for COVID-19 antigen detection were developed and commercially introduced in the market based on different formats of testing, with or without EUA from FDA. The antigen detection assays are mainly based on either lateral flow (LF) immune-chromatography or enzyme-linked immunosorbent assays (ELISA) regarding COVID-19 testing, primarily testing the antigen in respiratory specimens.

Lateral flow (LF) rapid diagnostic test (RDT)
The LF RDT is based on Antigen (Ag)-Antibody (Ab) binding. The test device includes a well for sample dispensing, and a paper-like nitrocellulose membrane composed of two lines: a control line that is coated with gold nano-particle-polyclonal anti-human globulins conjugates (assure test validity), and a test line that is coated with specific capture Abs [30]. The TAT of these tests ranges between 10-30 minutes.

Commercial LF kits (qualitative) are being marketed. Examples of these include: "COVID-19 Ag GICA Rapid" kit (manufactured by PCL, Korea) and claiming an accuracy of 85%. Another Korean kit is the CE (certification by European Union and Economic Area) – marked "Biocredit COVID-19 Ag" (manufactured in Korea by RapiGEN, Inc.) claiming a sensitivity of 92% and a specificity of 98%, and a CE-marked kit "BioEasy Diagnostic kit

for 2019-Novel Coronavirus (2019-nCoV)" (manufactured by Shenzhen BioEasy Biotechnology Co., China), claims to have a sensitivity and a specificity of 91.72% and 100%, respectively. Another model for LF is the CE-marked Belgian kit "COVID-19 Ag Respi Strip" (manufactured by CORIS BioConcept - Belgium) utilizes LF in dipstick. It is reported to have a viral detectability of 5x10exp3 pfu/mL and recombinant protein detectability of 0.25ng/mL. Moreover, the kit was validated in comparison with RT-PCR on two different populations revealing a sensitivity of 85.7%, a specificity of 100%, PPV of 100%, and NPV of 85.2%. Very Recently (May 11, 2020), the first FDA approval of rapid COVID-19 Ag detection test was granted to Quidel Corporation (San Diego, USA) "Sofia 2 SARS Antigen FIA". The test is based on LF immunofluorescent sandwich assay for the qualitative detection of nucleoprotein (NP) Ag utilizing dedicated Sofia 2 instrument. The claimed sensitivity and specificity were 80% and 100%, respectively, showing no cross reactivity with the common respiratory coronaviruses [https://www.fda.gov/media/137885/download]

ELISA based tests

ELISA is the other format of COVID-19 Ag detection assays which can be qualitative or quantitative. Most available kits are based on "Sandwich ELISA", where Micro-wells are coated with specific monoclonal Ab against viral Ag protein. The sample is added into the wells. If it contains Ag, it will react with the corresponding specific Ab, forming Ag-Ab complex. An enzyme (e.g. alkaline phosphatase, horseradish peroxidase) conjugated with a second Ab specific for the COVID-19 Ag will attach to the complex and the reaction is revealed by a color enzyme substrate. The color absorbance is measured and can be correlated with presence/absence of Ag in qualitative ELISA. Generally, the TAT for this type of assay takes 2-5 hours [5].

Few commercial ELISA kits for COVID-19 Ag detection are released to the market without US-FDA approval. For example, "SARS-CoV-2 Antigen ELISA Kit (DEIA 2020)" (manufactured by Creative Diagnostics-USA), is an example for quantitative ELISA tests that detect COVID-19 Nucleoprotein (NP) Ag. The sample could be human serum or plasma, and the claimed sensitivity of this test is 6.25ng/mL. Another example is the "COVID-19 Antigen ELISA Kit" (manufactured in China by Beijing Kewei Clinical Diagnostic Reagent Inc). It detects the viral Ag of COVID-19 qualitatively in nasal swabs, throat swabs, serum, or plasma samples, claiming a sensitivity of 98.7% and a specificity of 97.1%. Concerning cross reactions among these 7 antigen detection kits, only the CORIS BioConcept and the Biocredit kits noted weak cross reactions with some other coronaviruses.

In conclusion on rapid antigen-based testing

Rapid antigen-based diagnostic assays were introduced in anticipation to be similar in utility to the reference molecular tests, while alleviating their sophisticated and complex limitations. Though the test sensitivity and specificity remain to be refined to avoid false negative results, the rapid antigen testing is of value in helping control viral spread, in decision for returning to work, normalizing life and minimizing the apprehensiveness of individuals, as well as in unlocking mass testing capabilities. However, reliability of their performance remains in need of further validation in correlation with the clinical history.

RAPID IgG/ IgM SEROLOGIC TESTS FOR COVID-19

Introduction

Appropriate and accurate elucidation of the serodiagnostic features and immune responses of COVID-19 remain a priority for researchers as a possible strategy for detection. Mystery surrounding the pathogenesis, clinical and diagnostic features of the virus are attributed to its morbidity and sequelae. Very few guiding reports about the testing strategy are available regarding the laboratory diagnosis of this virus [4-6].

One would assume that infection with COVID-19, like any other infectious etiology, would stimulate the immune response by triggering mobilization of the T and B cells of the immune system, initiating an immune response from both cell-mediated immunity (CMI) and antibodies-mediated immunity respectively. The latter entails the production of specific immunoglobulins (Ig) of the IgM, IgG and IgA classes of antibodies. In the immune response to COVID-19 exposure, the evolution and role of CMI as a diagnostic or determinant of exposure remain unclear. The specific immunoglobulins, however, were reported mainly to be used as immune response indicators of exposure to the virus, and in certain situations can have diagnostic value [31].

Infectious viral dose and antibody response

The exact infectious viral dose (number of viruses) that can cause infection in humans and subsequently lead to the symptomatic or asymptomatic status remains to be determined. The justified rational of using face masks is to prevent or to minimize the infectious dose of the virus, thus allowing the immune response to handle without consequences.

Studies on the timing of antibody production due to infection indicated that it takes days to weeks to be reliably detectable. For example, Zhao et al. reported that in patients with post viral exposure, it took between 8 to 11 days for both specific IgM and IgG to be detected. Moreover, after the onset of symptoms, the positive rates of

detecting specific COVID-19 immunoglobulins (IgM, IgG, IgA) were revealed to range between 77.9% and 92.7% [32].

On the other hand, Guo et al. reported longer time (17-19 days) for the appearance of specific IgG and IgM among patients with acute COVID-19 infection (n = 285) [31]. In addition, they observed three types of seroconversion: simultaneous appearance of IgG and IgM (34%), IgM appearance earlier than IgG (27%), and IgG appearance earlier than IgM (39%). Moreover, they reported that in few cases of asymptomatic infection (n = 7), positive IgG and IgM were detected while the PCR was negative [31]. These thought-provoking findings require further verification since these observations were elicited from a small sample size.

Antigens used in serodiagnostic assays

The basis for most COVID-19 serological assays are on antibody detection against different viral antigens such as: immunogenic spike protein [especially the receptor binding domain (RBD) and/or NP], viral nucleocapsid proteins and developed recombinant antigens. Some of these share homology among other human coronaviruses. So, one has to be familiar with the antigen being used in the assay since difference in seroconversion is noted among these antigens. This is so because the onset of seropositivity was earlier for anti-RBD vs. anti-NP, for both IgG and IgM. In general, earlier seroconversion was seen for IgG vs. IgM for both anti-RBD and anti-NP [31].

Rapid diagnostic tests (RDTs) for antibody detection

To assess the generated humoral immune response, numerous commercial and laboratory-developed RDTs (mostly qualitative) have been introduced. Few products, however, have received FDA's EUA, while others are performing internal validation or lack appropriate ones.

Detailed information can be found through the following links: [FAQs on Diagnostic Testing for SARS-CoV-2.https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic302testing-sars-cov-2; and the Emergency Use Authorizations. https://www.fda.gov/medical304devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd305]. Updating the list of FDA approved or pulled out kits is an ongoing process.

These tests are designed to detect and identify different antibody classes (IgG, IgM, IgA or total antibodies) in individuals infected or exposed to COVID-19. Most tests use blood specimens (serum, plasma or whole blood) while few are geared to detect secretory IgA antibodies from saliva [32,33]. Examples of these commercial tests were cited by John Hopkins Center for Health security (found in the link below). It describes and categorizes tests as those approved for diagnostic use in the USA (n = 7), for diagnostic use in other countries (n = 9), for research or surveillance purposes only (n = 34), and tests that are still in development (n = 15).
[http://www.centerforhealthsecurity.org/resources/COVID-19/Serology-based-tests-for-COVID-19.html]

In the USA, the FDA granted EUA to tests, enabling their use in diagnostic laboratories. So far, only one test was granted approval by the USA FDA, namely the Cellex rapid, a lateral flow IgG and IgM test, where results are available in 15-20 minutes, once the blood is processed.

The main testing formats of these serodiagnostic tests are based on immunochromatographic LF immunoassay (also used as point of care test), ELISA, and chemiluminescent immunoassays (CLIA). These have rapid turnaround time (TAT), being cheaper and less complex to perform than the molecular tests, but not in lieu of the molecular tests [31,34].

ELISA antibody detection immunoassay

The ELISA test is similar in its procedure to that described in the antigen detection section above. It differs in that the micro-wells would be coated with specific Ag, and the enzyme is conjugated with a secondary Ab against the specific antibody being tested for in the patient's serum [5].

According to Roche, their antigen is the NP because it "provided the best specificity with a collection of pre-pandemic specimens." They say their upcoming package insert will state sensitivity of > 95% and specificity of 99.8%. It is an automated, 18 minute assay and is sold in packs of 200 tests. [https://www.roche.com/media/releases/med-cor-2020-05-03.htm].

Lateral flow format for antibodies detection

The rapid (10-15 minutes) LF test is similar in its procedure as that described under the antigen detection section above. However, instead of COVID-19 Abs being used, specific COVID-19 viral Ag proteins are coated so that the antibody in the patient sample (serum, plasma or whole blood), if present, would bind/react with the antigen forming Ag-Ab colored complex at the IgG, or IgM, or both lines and the control line [5].

Examples of EUA FDA approved automated assays include:

The Abbott SARS-CoV-2 IgG test detects COVID-19 IgG, qualitatively, in serum and plasma specimens based on using the Architect instruments based on chemiluminescence microparticle immune-assay, using microparticles precoated with COVID-19 NP Ag. The sample is incubated with microparticles precoated with specific

COVID-19 NP Ag. A conjugate anti-human IgG labeled with acridinium is added. Substrates (pre-trigger & trigger) are then added to yield chemiluminescence which reflects presence/absence of IgG. The claimed sensitivity for this kit is 100% at day 17 after symptom onset and day 13 after PCR positivity, and the specificity is 99.90%. It is CE-marked and has received FDA-EUA and has a TAT of about 29 minutes [35].
[https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance]

The Roche Elecsys Anti-SARS-CoV-2 assay uses Cobas instrument based on sandwich electrochemiluminescence for the qualitative detection of COVID-19 Ab in serum and plasma against NP Ag. The sample is mixed with two biotinylated specific recombinant Ags, one is labelled with ruthenium element and the other is unlabeled to form a complex. Subsequently, streptavidin-coated magnetic microparticles are added so that the complex becomes bound to solid phase via interaction between biotin and streptavidin. If positive for Ab, chemiluminescent emission is detected. The claimed sensitivity for this kit depending on days of onset of symptoms is 65.5% (0-6 days), 88.1% (7-13 days) and 100% (≥ 14 days), whereas the claimed specificity is 99.8%. It received FDA-EUA and has a TAT of about 18 minutes. [https://www.roche.com/media/releases/med-cor-2020-05-03.htm].

Cross reactivity

False positive COVID-19 testing was estimated to be around 2%, generated by antibodies present due to past or present infection with other human coronaviruses strains such as coronavirus HKU1, NL63, OC43, or 229E [36].

Sensitivity and specificity

A wide variation in the accuracy of sensitivity and specificity rates were reported among the marketed serodiagnosis RDT kits. For example, among 14 commercial kits manufactured in China, Korea and other countries that were proposed from vendors to AUBMC in Lebanon, the claimed sensitivity and specificity for IgG were 95%-100% and 85%-100%, respectively, and for IgM 88%-98% and 85%-100%, respectively. In the document from Johns Hopkins, it was noted that serology tests in development have a range of sensitivity (87% to 93%) and specificity (95% to 100%) [5]. However, in an interview with Embed, Dr. Gary Procop at Cleveland Clinic reported lower sensitivity (85.2%) for the ID Now kit based on testing 239 specimens known to contain the coronavirus. This is unacceptable as 14.8% of the tested patients would be called negative. More comforting for use, especially for accredited laboratories by the College of American Pathologists, is the first FDA approved RDT by Cellex which has a sensitivity of 93.8% and a specificity of 95.6%. [https://www.centerforhealthsecurity.org/our-work/publications/developing-a-national-strategy-for-serology-antibody-testing-in-the-US].

In the link below, FDA has summarized the expected performance characteristics of 11 serologic assays granted an EUA, assuming a prevalence of 5% for PPV and NPV calculations.
[https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance]

However, FDA is keeping an ongoing dynamic revision and update about commercial manufacturers of antobody tests: list of antibody tests.

Utility and potential use of RDTs

The utilities of the RDTs are essentially defined by the evolution and profile of the immune response post infection with COVID-19. Scattered information about the utility of RDTs were cited by different individuals, universities and societies [4-6].

One should keep in mind that these RDTs should be supported by the molecular PCR in many situations to achieve proper interpretation of results. Clarification of the different aspects, potential utility and facilitation of RDTs include:

- Assessment of community seroprevalence, contact tracing, surveillance and tracking spread of the virus nationwide. This is essential for epidemiologic studies and in defining the size and nature of the epidemic, guiding lockdown, reopening and integrating society decisions.
- Serologic assays are relevant to use when RT-PCR may be falsely negative such as in case of patients presenting to medical care with late complications of disease (viral shedding drops over time) [33]. The suggestion to use both methods concurrently was also noted in retrospective studies from China indicating that some COVID-19 infected patients were PCR negative, yet serology positive [31].
- At a hospital or medical center level, RDTs would be useful to test asymptomatic or pre-symptomatic staff: attending physicians, residents, nursing, as they might play an important role in transmission of infection to the high-risk population. This mass testing ability in a rapid TAT would guide and expedite the consideration of additional prevention measures, cohorting strategies and the decision to remove a suspect COVID-19 patient from isolation [37].
- RDTs would assess healthcare workers (HCWs) for post-infection. Test results can be helpful in decisions pertaining to return-to-work deploy-

ment, assuming there is a protective immunity by potent neutralizing antibodies.

- Identification of immune recovered patients to serve as convalescent plasma donors (based on quantitative antibody assays) for the potential of treating acutely ill patients or to provide passive immunity to non-immune health care workers on the front lines.

Having noted the above utilities of the RDTs, and though they are easy to perform, they require very cautious interpretation of results in correlation with the clinical condition and exposure history. Moreover, and prior to use, the performance characteristics of the test kit need to be properly validated according to established international guidelines.

Immunity - antibody neutralizing activity and dilemma

The serologic RDTs, though introduced to detect and determine IgG and IgM antibodies post infection, information about the ability of these antibodies to impart an "immunity" or protective status is evolving in this favor. Moreover, studies are still needed to learn about the antibody cut-off value, titer, or units associated with protective immunity or how long protective immunity may last.

Recent evidence from in-vivo experimental COVID-19 infected Rhesus Macaque primate (close to humans in their immune system), indicated that the immune response was protective against subsequent re-infection after resolution of a primary infection [38]. Such immune activity can also be reinforced by the in vitro study revealing neutralizing antibodies in sera of patients infected with COVID-19 [39]. Further supporting evidence can be elicited from earlier studies among high percentage (89%) of patients with SARS infection whose sera showed neutralizing antibodies for long duration (2 years) [40]. No doubt, all of these immune protective evidences empower the practical drive to the value of using plasma from recovered patients in the management of COVID-19 infection [41].

Based on the aforementioned evidence, one would wonder about those who question and doubt that these antibodies (recently labelled as "immunity passports") act as a signal of protective immunity against the COVID-19. Does it mean that this virus has a potent and rapid mutational capability to escape these antibodies? Are there different viral strains circulating globally, or is there a rapid deterioration and fading of these antibodies, defying their active persistence to defend against the re-exposure? In addition, one would wonder how would "herd immunity" be defined and achieved. Hopefully, the ongoing studies and research will find rapid answers to settle such difficult and valid questions.

Interpretation and meaning of serologic RDTs results

Testing and interpretation of the sero-RDTs should be done in coordination with the clinical history for proper assessment. This is necessary especially in case needed to advise about self-isolation, quarantine or hospital admission.

- Since these RDTs turn positive for specific COVID-19 IgM/IgG in around 8 to 11 days or more post viral exposure, they can show false negative results prior to this time in patients with symptomatic or early asymptomatic presentation [31]. Thus, this post viral exposure gap/delay in antibody production makes these RDTs not rule out infection during this period. In this situation, follow-up with a molecular diagnostic test should be considered to rule out infection in these individuals [21].
- A reactive/positive anti-COVID IgM antibody indicates recent acute infection or exposure. This warrants PCR testing for confirmation, and if positive can reflect the ability of transmission of the virus. If no symptoms, individuals would require self-isolation with symptoms monitoring. A quarantine or hospitalization would be required, if symptoms start appearing.
- A reactive/positive anti-COVID IgG antibody, indicates history of viral exposure (unknown time of recent or past), and needs to consider self-isolation for 14 days with symptoms monitoring. Referring to PCR if they become symptomatic. Some physicians may advise to determine if this is past (asymptomatic) or recent exposure by performing PCR to confirm. If PCR turns positive it indicates that the person can still shed and transmit the virus. If PCR is negative, it can reflect "immunity" against the virus, indicating asymptomatic or resolution of infection.
- Both IgG and IgM could show reactive/positive results simultaneously, and PCR would be considered especially if a patient needs admission to hospital.
- A nonreactive/negative anti-COVID IgM and IgG indicates either no exposure and no need for PCR, or an early exposure (asymptomatic) if contact is anticipated in a duration of less than 12 days where period during which a delay in antibody formation is expected, thus warranting PCR test to exclude early asymptomatic infection. If the latter turns out negative then no exposure to the virus is determined, while if it turns out positive then this would indicate recent exposure and possibility of trans-

mission of the virus. Bear in mind the possible negative findings due to immunosuppressed patients.

In conclusion on rapid antibody-based testing

Antibody-based testing is essentially targeted to determine the person's exposure to COVID-19 infection and its extent in the community. These rapid assays are also well suited in their diversified utility to help control viral infection and minimize apprehensiveness of individuals. It is of essential essence to take into consideration the need for appropriate interpretation of their results in conjunction with the clinical picture, risk factors of exposure and resorting to molecular assays when warranted.

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