

Review

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Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence

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Abstract: The global coronavirus disease 2019 (COVID-19) has presented major challenges for clinical laboratories, from initial diagnosis to patient monitoring and treatment. Initial response to this pandemic involved the development, production, and distribution of diagnostic molecular assays at an unprecedented rate, leading to minimal validation requirements and concerns regarding their diagnostic accuracy in clinical settings. In

addition to molecular testing, serological assays to detect antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are now becoming available from numerous diagnostic manufacturers. In both cases, the lack of peer-reviewed data and regulatory oversight, combined with general misconceptions regarding their appropriate use, have highlighted the importance of laboratory professionals in robustly validating and evaluating these assays for appropriate clinical use. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Task Force on COVID-19 has been established to synthesize up-to-date information on the epidemiology, pathogenesis, and laboratory diagnosis and monitoring of COVID-19, as well as to develop practical recommendations on the use of molecular, serological, and biochemical tests in disease diagnosis and management. This review summarizes the latest evidence and status of molecular, serological, and biochemical testing in COVID-19 and highlights some key considerations for clinical laboratories operating to support the global fight against this ongoing pandemic. Confidently this consolidated information provides a useful resource to laboratories and a reminder of the laboratory's critical role as the world battles this unprecedented crisis.

Keywords: biochemical monitoring; COVID-19; SARS-CoV-2; serology testing; molecular testing.

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Background

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a newly discovered coronavirus, identified as "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) [1]. As of May 11, 2020, there have been more than 4 million confirmed cases of COVID-19 and over 280,000 deaths across 187 countries. The global answer to this major pandemic from initial diagnosis and treatment to epidemiological surveillance hinges on clinical

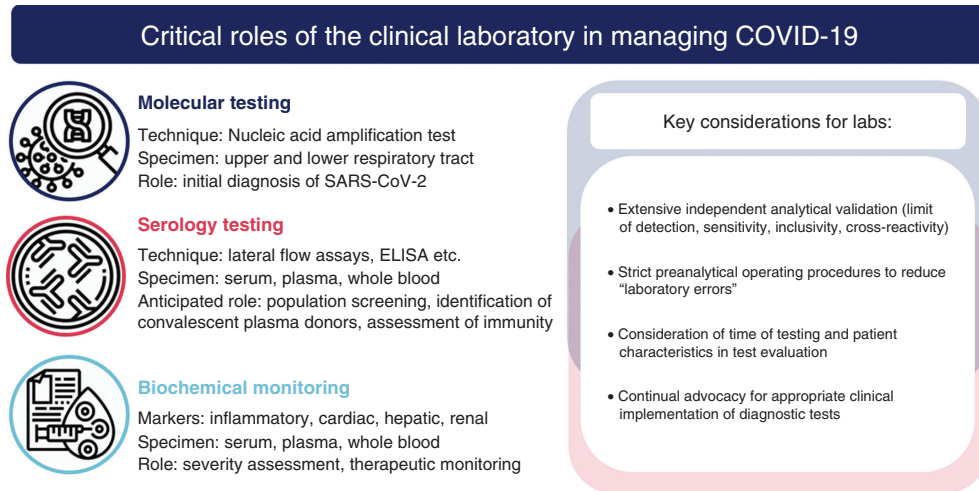


Figure 1: Overview of the key roles of the clinical laboratory in managing COVID-19.

laboratory testing. Importantly, the expertise of laboratory professionals is critical in robustly validating diagnostic assays to ensure sufficient analytical performance in support of ongoing healthcare efforts (Figure 1). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Task Force on COVID-19 has been established to support clinical laboratories and provide recommendations based on the best available evidence on biosafety as well as summarize key information regarding laboratory testing for diagnosing, prognosticating, and monitoring COVID-19. This review summarizes the latest evidence in molecular, serology, and biochemical testing in COVID-19 and highlights areas in urgent need of improvement and support.

Molecular testing

The importance of rapid and reliable molecular testing for the initial diagnosis of SARS-CoV-2 infection is well recognized and described, vitally contributing to case identification, isolation, contact tracing, and rationalization of infection control measures [2]. As such, both the diagnostics industry and clinical laboratories have been rapidly developing and validating different molecular tests for the detection of SARS-CoV-2. Due to the relatively short research and development time for both lab-based and point-of-care (POC) molecular assays, in many cases uncertainties regarding their clinical accuracy and sensitivity persist.

Nucleic acid amplification tests (NAATs) are currently the gold standard for diagnosing suspected cases of COVID-19 [3]. The most common type of NAAT applied

in authorized molecular assays is real-time reverse-transcription polymerase chain reaction (rRT-PCR), which is used by both the Centers for Disease Control and Prevention (CDC)-developed assay and the World Health Organization (WHO)-endorsed assays [2, 4, 5]. Isothermal nucleic acid amplification (i.e. reverse transcription loop-mediated isothermal amplification, transcription-mediated amplification, and CRISPR-based assay) represents the second most common type of NAAT used in authorized molecular assays [2]. However, very few authorized assays utilize these methods, including the ID NOW COVID-19 (Abbott Diagnostics Scarborough, Inc.) and the iAMP COVID-19 Detection Kit (Atila BioSystems, Inc.).

Preanalytical considerations

Specimen type

The preferred specimen type for molecular testing depends on patient characteristics and assay specifications. Both upper and lower respiratory tract specimens collected during the acute phase of infection are deemed suitable for detection of SARS-CoV-2 with NAATs.

Upper respiratory specimens include nasopharyngeal (NP), oropharyngeal (OP, i.e. throat), nasal mid-turbinate (NMT), and anterior nares swabs, along with NP wash/aspirate or nasal wash/aspirate. Interestingly, Zhang et al. recently showed that 100% sensitivity of combined OP and NP swabs can only be achieved when as many as five consecutive OP and NP swabs are collected on consecutive days [6].

Lower respiratory tract specimens include sputum, bronchoalveolar lavage (BAL), tracheal aspirate, pleural fluid, and lung biopsy. The CDC recommends using upper respiratory specimens for initial diagnostic testing, likely for logistical simplicity and lower invasiveness compared to all but sputum [7]. Lower respiratory tract specimens are more suitable in hospitalized patients (e.g. BAL) or patients presenting with a productive cough (e.g. sputum). As expected, BAL and sputum have been shown to have the highest positive rates for viral detection via rRT-PCR (over 90%), compared to other sample types in the literature [8]. Similarly, several studies have reported that nasal swabs have higher positivity rates than throat swabs (i.e. typically ~60% vs. ~40%) [8–10]. Higher viral load and a longer mean duration of viral detection in respiratory samples correlate with disease severity. However, high variability is observed [11].

Even though the NP swab is viewed as the gold standard sample for detection of SARS-CoV-2 using molecular tests, a perceived drawback is the requirement for healthcare professionals to obtain the specimen, typically necessitating the patient visit a hospital or other collection site. The US Food and Drug Administration (FDA) recently authorized a home collection kit for nasal swabs. Kit purchasers collect the sample themselves and mail it back to the laboratory for testing. This approach to sample collection may seem appealing, but no experiments have yet been performed to demonstrate equivalence between nasal specimens collected by healthcare professionals and those collected by patients themselves at home. Sullivan et al. suggest a protocol for this kind of validation [12].

Every test result is only as good as the sample from the patient. NP samples are difficult to obtain and often momentarily painful for the patient. Most patients, even with superb instruction, will not acquire adequate samples and thus the rate of false negatives for such testing will potentially be high. Salivary samples that will simplify sample collection have recently been proposed [13, 14]. However, the suitability and impact of this sample type on assay sensitivity is unknown. Viral load in blood, urine, and stool is less frequently reported [15]. However, when present, viremia strongly correlates with clinical severity [16, 17].

As identified by Lippi et al., preanalytical issues include inadequate procedures for collection, handling, transport and storage of the specimens (especially OP and NP swabs), as well as inadequate sample material in terms of poor quality or volume [18]. Interfering substances present in the specimens, sample contamination, and pipetting errors while preparing the material for testing also present preanalytical issues.

Time of testing

In addition to the aforementioned preanalytical considerations, the diagnostic testing window is perhaps one of the most important factors impacting test sensitivity. False negatives may be caused by low viral loads in the early and late stages of infection. In a virologic assessment of hospitalized patients, maximum viral load in throat swabs was observed in the first 5 days after symptom onset [15]. Similarly, viral loads have been reported to be highest in posterior oropharynx saliva samples within 7 days after symptom onset [19]. The median (interquartile range) period between symptom onset and a negative RT-PCR result has been reported as 20 [17–24] days in a population of 216 hospitalized patients [9].

Analytical considerations

Gene target

Molecular assays used to diagnose SARS-CoV-2 are designed to detect specific target viral genes and regions, including the spike (S), envelope (E), and nucleocapsid (N) proteins, which represent three of the four proteins that structurally constitute the virus, as well as the RNA-dependent RNA polymerase (*RdRp*) gene and the Open Reading Frame 1ab (*ORF1ab*) region. Thus, viral recombination or mutation may represent an analytical issue. Already, evidence of active viral recombination of SARS-CoV-2 has been shown [20]. According to a study that is not yet peer-reviewed, Korber et al. have identified 14 amino acid mutations in the S protein of SARS-CoV-2. Many more are the synonymous mutations observed at the nucleotide level (<https://www.gisaid.org/>). Such recombinations and mutations may compromise the accuracy of RT-PCR results, due to mismatches between the RNA sequence of the specimen and the primer and probes of the assay, which can lead to false negatives [21]. Recent studies have correlated some emerging mutations with different epidemiological features, including higher transmissibility and a more severe clinical outcome [22]. If confirmed by broader epidemiological studies, the refinement of the current assays to specifically detect clinically important mutations may provide valuable information for the management of COVID-19. Lack of harmonization between primer and probe sets limits comparison of assay performance between different platforms. Further analytical issues include inadequate assay validation, instrument malfunction, inaccurate cut-off definition, result misinterpretation, and others [18].

Clinical performance of molecular tests compared to chest CT

Most regulatory agencies require some level of analytical (e.g. limit of detection [LoD], inclusivity, and cross-reactivity evaluations) and clinical validation prior to authorization [23]. Despite the recommended validation procedures, SARS-CoV-2 diagnostic assays present the potential for reduced sensitivity and false-negative results as in the rush to put testing to use, evaluations have not been completed as well as usually done by laboratory professionals.

There are few studies comparing the diagnostic sensitivity of molecular tests to chest CT, which is considered an ancillary method in most regions. Specifically, Fang et al. in Wuhan, China first demonstrated significantly lower sensitivity of RT-PCR in OP and NP swabs (71%) compared to chest CT (98%) in a small retrospective analysis of 51 patients [24]. In a similar examination of 1014 cases, Ai et al. observed lower sensitivity in RT-PCR in the same specimens compared to chest CT. In fact, 48% of patients with negative RT-PCR results for SARS-CoV-2 were deemed as highly likely cases based on CT imaging [25]. Other reports with a higher prevalence of milder cases have determined comparable sensitivity rates for RT-PCR on OP and NP specimens and chest CT [26]. However, these reports lack information about the sensitivity of the used RT-PCR assays as well as details of the time intervals between symptom onset and sample collection for RT-PCR testing, especially in cases of negative results. Thus, interpretation of the observations remains challenging, in particular as a few reported intervals between chest CT images and corresponding RT-PCT suggest timeframes of > 1 week between symptom onset and RT-PCR [25]. As Wölfel et al. had shown, viral loads in throat swabs of some patients may become undetectable after this period [15].

In addition to comparison with chest CT, some studies have compared commercially available RT-PCR assays to ‘gold standard’ assays recommended by the WHO and CDC and against each other. These studies are increasingly important as many labs start to adopt commercial tests for clinical practice [27–32]. For example, Poljak and colleagues [28] compared the commercial Roche Cobas SARS-CoV-2 test to the assay described by Corman et al. [33] and found good (>98%) overall agreement. Zhen et al. also compared four molecular tests (New York SARS-CoV-2 Real-time Reverse Transcriptase [RT]-PCR Diagnostic Panel [Modified CDC], the Simplexa COVID-19 Direct [Diasorin Molecular], GenMark ePlex SARS-CoV-2 assay [GenMark], and the Hologic Panther Fusion® SARS-CoV-2 assay [Hologic]), demonstrating variable LoDs

(analytical sensitivity), but rather similar clinical performance [32]. In addition, Uhteg et al. reported similar analytical performance among three different assays (the RealStar® SARS-CoV-2 RT-PCR, ePlex® SARS-CoV-2, and the CDC COVID-19 RT-PCR tests) [29], while Lieberman et al. compared four commercially available assays (Cepheid, DiaSorin, Hologic Panther, and Roche Cobas) and demonstrated some variability in analytical sensitivity [30]. Importantly, a recent report also suggests increased potential in false-negative rates at the claimed lower limit of detection (LLoD) in six commercial assays [31]. Whether molecular assays are carried out at the POC or using automated clinical laboratory assays, the quality of these tests is paramount and more studies would be needed to further elucidate the relative performance of newly emerging tests. With variable study conclusions, it is important to consider both the analytical and preanalytical factors that impact the performance of molecular testing.

Regulatory perspectives and assay authorization

The WHO maintains a list of molecular assay protocols [5], which includes the Charité (Germany) assay described by Corman et al. [33], China Center for Disease Control and Prevention (China CDC) assay, United States Centers for Disease Control and Prevention (US CDC) assay, Institut Pasteur (France) assay, National Institute of Infectious Disease (Japan) assay, HKU (Hong Kong) assay, and the National Institute of Health (Thailand) assay. The WHO has also issued an Emergency Use List (EUL) pipeline and maintains a comprehensive list of assays authorized by different regulatory bodies [34]. The European Commission refers to the European Centre for Disease Prevention and Control (ECDC) and the WHO for guidance on molecular testing, although they have published their own directive regarding authorization, which is denoted when a product is ‘Community European (CE)-marked’. Unfortunately, a comprehensive list of ‘CE-marked’ assays is not available at this time. In the US, only assays that have received Emergency Use Authorization (EUA) from the FDA are permitted for use as diagnostic tests. Similarly, authorization from Health Canada must be received before assays can be used for diagnostic purposes in the country. Other countries, including Australia, Singapore, China, Korea, Russia, and Brazil, also maintain lists of authorized assays. In all cases, laboratory-based assays greatly outnumber the available POC assays on the market; however the latter

are beginning to become more available in response to increasing demand for rapid test results. While the use of rapid POC assays for molecular diagnosis of COVID-19 is highly attractive, independent validation data are urgently needed prior to clinical use at locations beyond healthcare institutions. A list of POC NAATs that are authorized for use in the US and Canada is presented in Table 1.

Clearly, there is still much work to be done in the area of molecular testing in COVID-19, particularly as new commercial assays continue to be developed and/or modified. Furthermore, a better understanding is required during which phase of COVID-19 the application of NAAT testing in which kind of sample type is most beneficial for patient management. Thus, based on current evidence, an initial negative NAAT result does not rule out SARS-CoV-2 infection due to potential pre-analytical and analytical issues or time of testing. Accordingly, combining NAAT testing with other methods may be key to improved patient diagnosis and therapy monitoring. Finally, for reliable test results the availability and timeliness of provision of external quality assessment (EQA) programs are key, as they are necessary to identify systematic testing errors, provide objective evidence of testing quality, and compare laboratory performance. The Royal College of Pathologists Australasia Quality Assurance Programs (RCPAQAP) first produced a Proficiency Testing Program (PTP) for the detection of SARS-CoV-2 by PCR in early March 2020; the participants were a selection of Australian and NZ laboratories that were performing testing for COVID-19 at the time. A second round of proficiency testing involving many more laboratories is currently under way, with a third round planned for later in the year. The WHO has a global influenza proficiency testing scheme that will include SARS-CoV-2 in a testing panel targeting national reference laboratories and this is produced by the Centre for Health Protection (CHP) in Hong Kong. That panel is expected to be dispatched soon. Quality Control for Molecular Diagnostics (QCMD) in Scotland has a pilot EQA scheme for coronaviruses, the Coronavirus Outbreak Preparedness EQA Pilot Study, which is currently open and will also be dispatched soon. The ECDC is currently organizing EQA programs. In addition, the National Center for Clinical Laboratories in China has also started conducting EQA programs among 855 laboratories. Two Canadian-based companies, Microbix Biosystems Inc. and Oneworld Accuracy, also recently announced a joint collaboration to provide a global EQA program. In lieu of these programs, more independent validation data are urgently needed.

Serology testing

Serology testing for COVID-19 can be defined as analysis of plasma, serum, or whole blood for the detection of antibodies, especially immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), that are specific for SARS-CoV-2 antigens, including the spike glycoprotein and nucleocapsid protein [2]. Testing methodologies vary from rapid diagnostic tests used at the POC (disposable immunochromatographic lateral flow assays), to enzyme-linked immunosorbent assays (ELISA) or chemiluminescent immunoassays run on fully automated clinical laboratory instruments, as described elsewhere [2].

There is currently high demand for specific and sensitive serology assays for detecting COVID-19 antibodies. Serology testing is anticipated to play an important role in the diagnosis of past COVID-19 infection, to better assess the prevalence of infection at a population level. Serology could also be of help in confirming suspected cases, especially in patients with either mild to moderate illness or tested in the late phase of COVID-19, not detected with molecular assays [35]. It is also estimated to be essential in the identification of potential convalescent plasma donors, as well as continued immunological/epidemiological surveillance and in monitoring immune responses of future COVID-19 illness. However, with the rapid emergence of several immunoassays from diagnostic manufacturers, their accuracy, clinical utility, and value remain largely uncharacterized.

Preanalytical considerations

Time of testing

Similar to molecular testing, the timeframe of serological testing and the performance characteristics of antibody tests are closely linked. Accumulating data suggest that seroconversion occurs approximately 7–14 days after symptom onset [36–38]. One publication using an ELISA method in serial plasma samples from hospitalized COVID-19 patients showed median seroconversion for total immunoglobulins, IgM, and IgG on day 11, day 12, and day 14, respectively [37]. Other studies using an internally developed ELISA report much earlier detection of IgM with one report indicating detectable IgM in 85% of COVID-19-confirmed patients 1–7 days after symptom onset [39]. Notably, IgA response develops early, coinciding with that of IgM, peaks after 18–21 days, and appears to be even stronger and more persistent than the IgM

Table 1: Molecular point-of-care assays authorized in the US and Canada.

Company (assay name)	Gene target	Accepted specimen type	Method	Analytical validation and clinical performance	Authorization for clinical use
Abbott Diagnostics Scarborough, Inc. (<i>ID NOW COVID-19</i>)	RdRp	Nasal, throat, NP	– Isothermal nucleic acid amplification	<ul style="list-style-type: none"> – LoD: 125 copies/mL (reference SARS-CoV-2 material) – Inclusivity: RdRp = 100% for all sequences with no mismatches identified – Cross-reactivity (<i>in silico</i> analysis): no significant cross-reactivity – PPA (contrived, NP): 100% (20/20) at 2X LoD and 100% (10/10) at 5X LoD – NPA (NP): 100% (30/30) – LoD: 250 copies/mL (reference SARS-CoV-2 material) and 0.0100 PFU/mL (SARS-CoV-2 RNA/strain USA_WA1/2020) – Inclusivity: some mismatches found, but >80% homology for both targets 	– US (FDA)
Cepheid (<i>Xpert Xpress SARS-CoV-2</i>)	N2, E	NP, OP (i.e. throat), nasal, mid-turbinate swab, nasal wash/aspirate	– rRT-PCR	<ul style="list-style-type: none"> – Cross-reactivity (<i>in silico</i> analysis): E primers/probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus – PPA (contrived, NP): 100% (20/20) at 2X LoD, 100% (5/5) at 3X LoD, and 100% (5/5) at 5X LoD – PPA (live virus, NP): 100% (20/20) at 2X LoD, 100% (5/5) at 3X LoD, and 100% (5/5) at 5X LoD – NPA (NP): 100% (35/35) and 100% (30/30) – LoD: 100 copies/mL (reference SARS-CoV-2 material) and 200 copies/mL (SARS-CoV-2 RNA/strain USA_WA1/2020) – Inclusivity: some mismatches found, but >80% homology for both targets – Cross-reactivity (<i>in silico</i> analysis): primers/probes are not specific for SARS-CoV-2 and may detect SARS-CoV – Cross-reactivity (testing organisms): no significant cross-reactivity – PPA (contrived, throat/nasal): 100% (20/20) at 2X LoD, 100% (7/7) at 5X LoD, 100% (2/2) at 10X LoD, and 100% (1/1) at 50X LoD – NPA (throat/nasal): 100% (30/30) 	– US (FDA) – Canada (Health Canada) – Australia – Singapore – Philippines – Brazil
Mesa Biotech, Inc. (<i>Accula SARS-CoV-2 Test</i>)	N	Throat, nasal	– RT-PCR	<ul style="list-style-type: none"> – Cross-reactivity (<i>in silico</i> analysis): primers/probes are not specific for SARS-CoV-2 and may detect SARS-CoV – Cross-reactivity (testing organisms): no significant cross-reactivity – PPA (contrived, throat/nasal): 100% (20/20) at 2X LoD, 100% (7/7) at 5X LoD, 100% (2/2) at 10X LoD, and 100% (1/1) at 50X LoD – NPA (throat/nasal): 100% (30/30) 	– US (FDA)

NP, nasopharyngeal; OP, oropharyngeal; LoD, limit of detection; PPA, positive percent agreement; NPA, negative percent agreement. Information accessible at: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations>.

response [40]. Overall, the diagnostic use of serological testing in the acute phase of infection remains to be clearly demonstrated. At the present time, it is unknown how long antibodies persist following infection, and whether all antibodies produced are capable of neutralizing the virus. Ideally, the sensitivity of available assays should be evaluated in serially collected samples varying in days after symptom onset.

Patient characteristics

Another important consideration in serology testing is the patient population in which the test has been evaluated. Recent data suggest that patients with severe COVID-19 have higher IgG and IgM titers compared to those with non-severe COVID-19 [41]. Many manufacturers have evaluated the sensitivity and specificity of their assay using serum or plasma samples collected from severe COVID-19 patients. The Norwegian Organization for Quality Improvement performed a limited evaluation of the diagnostic performance of 11 rapid serology tests in three separate clinical groups: (1) hospitalized patients, (2) recovered patients with previously PCR-confirmed COVID-19 who did not require hospitalization, and (3) patients with suspected COVID-19 presenting at an emergency room. In their study, true positivity rates were the highest in hospitalized patients and the lowest in patients with suspected COVID-19. Moreover, additional findings suggest that some proportion of asymptomatic carriers were seronegative likely due to low viral load [38, 42, 43]. More robust studies are needed to further evaluate the sensitivity of these assays in different clinical populations, particularly if they are to be used to detect past infection in asymptomatic individuals.

Analytical considerations

Antibody target

Currently available serological assays rely on the detection of different antibodies, including total antibody, IgG, IgM, and IgA. Classical immune response to viral pathogens generally involves IgM production first, often accompanied by emergence of IgA, and then followed by a shift toward IgG production [44, 45]. In COVID-19, current evidence is conflicting with some groups concluding IgM is produced first [39] and others suggesting IgM and IgG production occur simultaneously, similar

to what has been observed in other SARS-associated coronaviruses [46, 47]. Nevertheless, IgM is unlikely to play the primary role in COVID-19 antibody testing due to traditional specificity challenges associated with high false-positive rates [48]. IgG is a longer lasting antibody associated with potential viral neutralizing activity [49, 50]. Many manufacturers have therefore focused their efforts on developing immunoassays against IgG rather than IgM. IgA testing provides a clinically useful alternative. These antibodies are secreted at the surface of body mucosae and their detectability and titer in serum or plasma may reflect the immune function of mucous membranes [40]. Further longitudinal studies on antibody response to COVID-19 are required to understand the dynamics of respective immunoglobulins and their role in managing SARS-CoV2 infections.

Cross-reactivity

A common concern in serological testing is cross-reactivity, an aspect magnified by the evidence that SARS-CoV-2 and SARS-CoV-1 share almost 80% genetic identity [51, 52] and common proteins show structural homology with SARS-CoV-1 which caused SARS in 2002–2003 [53]. The potential for cross-reactivity in COVID-19 is particularly significant in adult populations given previous seroprevalence studies indicating that over 90% of adults have antibodies to the common circulating coronaviruses, even if homology with these strains is lower [49, 54, 55]. Preliminary claims in manufacturer package inserts are encouraging, with most companies indicating no cross-reactivity. However, the number of viral strains evaluated varies from manufacturer to manufacturer, and more peer-reviewed data are urgently needed.

Clinical performance of serological tests compared to virus neutralization assays

It is important to highlight that current serology assays available from commercial diagnostic manufacturers do not provide any definitive information regarding patient immunity. For this analysis, lab-based neutralization assays in a cell culture system are needed to determine the presence of active antibodies and relative protection against future infection. To date, our understanding of whether the presence of antibodies confers protective immunity remains incomplete. Preliminary studies have shown evidence of neutralizing antibodies in serum of recovered middle-aged and elderly patients, suggesting

an age-related innate immune response [56]. The demonstration of antibody neutralization is not only important for determining protective immunity, but also for feasibility and efficacy of therapeutic treatment with convalescent (“hyper-immune”) plasma [50, 57]. Currently, neutralizing antibodies from convalescent plasma donors have been used to treat COVID-19 in non-randomized settings [56]. Specifically, Shen and colleagues completed an uncontrolled case series of five critically ill patients with COVID-19 that had progressed to acute respiratory distress syndrome (ARDS) [58]. A larger study in 10 severe adult cases demonstrated that one dose of convalescent plasma could lead to clinical improvement within 3 days [59]. In either study, administration of convalescent plasma resulted in general albeit non-specific improvement in clinical status [58, 59]. These preliminary findings are encouraging for the safety and effectiveness of convalescent plasma treatment. However, they are not randomized trials and warrant further study to ensure that antibody-dependent enhancement of viral infection does not occur [60].

Regulatory perspectives and assay authorization

Serology assays currently available from diagnostic manufacturers vary significantly in their methodology, antibody target, and acceptable specimen type [49]. More than 90 serology assays have been submitted to the FDA, with 12 receiving EUA (as of May 11, 2020). However, unlike molecular COVID-19 testing, the FDA did not require EUA for the clinical application of serological testing until recently [61]. Table 2 summarizes the characteristics of available commercial serological assays that have been granted EUA. While these assays have been marketed widely, there is currently minimal peer-reviewed data on their performance in clinical settings. Thus, our knowledge relies almost completely on manufacturer claims and limited peer-reviewed and some non-peer-reviewed data [62–64], limited by sample size, patient characteristics, and sampling time. The lack of peer-reviewed data and regulatory oversight combined with general misconceptions regarding the appropriate uses of serology testing in COVID-19 have highlighted the crucial role of the clinical laboratory in robustly validating these tests to ensure sufficient analytical and clinical performance.

Overall, the diagnostic performance of serology assays has not been systematically evaluated, and more data are urgently needed to support their clinical utility in different settings. As Farnsworth et al. recently emphasized,

in low prevalence settings, even immunoassays with excellent specificity can lead to insufficient positive and negative predictive values, and could misinform practice and policy [65]. Algorithms to confirm an initial positive assay result are common practice in transfusion medicine, usually employing alternative assays and methods like immunoblots, and might be valuable in COVID-19 in low prevalence settings to lower falsely positive result. It will be up to laboratory professionals to advocate for rigorous validation of these tests in the setting they will be clinically used, and then appropriate application based on the findings.

Biochemical monitoring in severe COVID-19

The role of the clinical laboratory in this crisis extends beyond initial diagnosis and epidemiological surveillance. Routine biochemical, hematological, and immunochemical laboratory testing is essential in assessing disease severity, selecting appropriate therapeutic options, and monitoring treatment response. As the number of confirmed COVID-19 cases continues to escalate globally, the laboratory abnormalities associated with higher disease severity are becoming increasingly clear (Figure 2).

Inflammatory biomarkers

Several inflammatory biomarkers have been implicated in severe COVID-19, suggesting an immunochemical profile consistent with the so-called “cytokine storm”. In brief, elevation of proinflammatory cytokines, particularly interleukin (IL)-6 and tumor necrosis factor- α (TNF- α), has been observed in patients with severe disease and also found to be significantly associated with mortality [66–69]. It is important to note that proinflammatory cytokines appear to be not solely biomarkers, but also causative factors in COVID-19 progression and mortality. Although cytokine measurement is not common in clinical laboratory practice, surrogate biochemical markers of inflammation including ferritin, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) have all been implicated in severe COVID-19 and can be useful in assessing disease severity [67–69]. Higher levels of procalcitonin have also been shown in severe COVID-19, suggesting onset of bacterial co- or supra-infection in critically ill patients [70]. In addition to biochemical markers of inflammation, hematological findings suggest that lymphopenia,

Table 2: Characteristics and performance of serological assays provisionally approved by the FDA under EUA.

Company (assay name)	Antibody target	Accepted specimen type	Analytical method	Analytical validation and clinical performance
Abbott (<i>Abbott Architect SARS-CoV-2 IgG</i>)	IgG	Serum and plasma (heparin, EDTA, citrate)	<ul style="list-style-type: none"> – Qualitative – Chemiluminescent microparticle immunoassay 	<ul style="list-style-type: none"> – Cross-reactivity: cytomegalovirus (CMV) IgG – Sensitivity: 100% (88/88) – Specificity: 99.6% (1066/1070) – PPV at prevalence = 5%: 92.9% – NPV at prevalence = 5%: 100%
Autobio (<i>Anti-SARS-CoV-2 Rapid Test</i>)	IgM and IgG	Serum and plasma (EDTA, heparin, citrate)	<ul style="list-style-type: none"> – Qualitative – Lateral flow (one-step capture method) 	<ul style="list-style-type: none"> – Cross-reactivity: none reported – Sensitivity: 88.1% (357/405) – Specificity: 99% (309/312) – PPV at prevalence = 5%: 82.9% – NPV at prevalence = 5%: 99.4%
Bio-Rad Laboratories (<i>Platelia SARS-CoV-2 Total Ab</i>)	Total Ab	Serum and plasma (EDTA)	<ul style="list-style-type: none"> – Qualitative – One-step antigen capture format, enzyme-linked immunosorbent assay 	<ul style="list-style-type: none"> – Cross-reactivity: none reported – Sensitivity: 92.2% (47/51) – Specificity: 99.6% (684/687) – PPV at prevalence = 5%: 91.7% – NPV at prevalence = 5%: 99.6%
Cellex, Inc. (<i>qSARS-CoV-2 IgG/IgM Rapid Test</i>)	IgM and IgG	Serum, plasma (EDTA, citrate), or whole blood (venous)	<ul style="list-style-type: none"> – Qualitative – Lateral flow immunoassay 	<ul style="list-style-type: none"> – Cross-reactivity: none reported – Sensitivity: 93.8% (120/128) – Specificity: 96% (240/250) – PPV at prevalence = 5%: 55.2% – NPV at prevalence = 5%: 99.7%
Chembio Diagnostic Systems, Inc. (<i>DPP® COVID-19 IgM/IgG System</i>)	IgM and IgG	Serum, plasma (EDTA, heparin), and whole blood (venous and capillary)	<ul style="list-style-type: none"> – Qualitative – Lateral flow (rapid immunochromatographic test) 	<ul style="list-style-type: none"> – Cross-reactivity: human coronavirus 229E and human coronavirus HKU1 – Sensitivity: 93.5% (29/31) – Specificity: 94.4% (118/125) – PPV at prevalence = 5%: 46.8% – NPV at prevalence = 5%: 99.6%
DiaSorin (<i>LIAISON® SARS-CoV-2 S1/S2 IgG</i>)	IgG	Serum and plasma (heparin and EDTA)	<ul style="list-style-type: none"> – Qualitative – Chemiluminescent immunoassay 	<ul style="list-style-type: none"> – Cross-reactivity: anti-HBV, rheumatoid factor, anti-influenza A – Sensitivity: 97.6% (40/41) – Specificity: 99.3% (1082/1090) – PPV at prevalence = 5%: 88% – NPV at prevalence = 5%: 99.9%

Table 2 (continued)

Company (assay name)	Antibody target	Accepted specimen type	Analytical method	Analytical validation and clinical performance
EUROIMMUN (<i>Anti-SARS-CoV-2 ELISA IgG</i>)	IgG	Serum or plasma (heparin, EDTA, and citrate)	<ul style="list-style-type: none"> – Qualitative – High throughput ELISA 	<ul style="list-style-type: none"> – Cross reactivity: <i>Chlamydomytila pneumoniae</i> (IgG), SARS-CoV(-1) infection (2020), ANA, other autoantibodies, Mycoplasma (IgM/IgG), respiratory syncytial virus (RSV), acute, severe bacterial pneumonia with high concentrations of procalcitonin – Sensitivity: 90% (27/30) – Specificity: 100% (80/80) – PPV at prevalence = 5%: 100% – NPV at prevalence = 5%: 99.5% – Cross-reactivity: none reported – Sensitivity: 92.5% (37/40) – Specificity: 100% (74/74) – PPV at prevalence = 5%: 100% – NPV at prevalence = 5%: 99.6% – Cross-reactivity: none reported – Sensitivity: 83.3% (30/36) – Specificity: 100% (400/400) – PPV at prevalence = 5%: 100% – NPV at prevalence = 5%: 99.1% – Cross-reactivity: none reported – Sensitivity: 87.5% (42/48) – Specificity: 100% (407/407) – PPV at prevalence = 5%: 100% – NPV at prevalence = 5%: 99.3% – Cross-reactivity: West Nile virus – Sensitivity: 88% (95/108) – Specificity: 98.8% (428/433) – PPV at prevalence = 5%: 79.4% – NPV at prevalence = 5%: 99.4% – Cross-reactivity: none reported – Sensitivity: 100% (29/29) – Specificity: 99.8% (5262/5272) – PPV at prevalence = 5%: 96.5% – NPV at prevalence = 5%: 100%
Mount Sinai Hospital Clinical Laboratory (<i>Mt. Sinai Laboratory COVID-19 ELISA Antibody Test</i>)	IgG	Serum and plasma	– High throughput ELISA	
Ortho Clinical Diagnostics (<i>VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total</i>)	Total Ab	Serum and plasma (EDTA)	<ul style="list-style-type: none"> – Qualitative – Immunometric 	
Ortho Clinical Diagnostics (<i>VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG</i>)	IgG	Serum	<ul style="list-style-type: none"> – Qualitative – Chemiluminescent immunoassay 	
Wadsworth Center, New York State Department of Health (<i>New York SARS-CoV Microsphere Immunoassay for Antibody Detection</i>)	Total Ab	Serum	– High throughput ELISA	
Roche (<i>Elecsys Anti-SARS-CoV-2</i>)	Total Ab	Serum and plasma (EDTA and heparin)	<ul style="list-style-type: none"> – Qualitative – Electrochemiluminescence immunoassay 	

PPV, positive predictive value; NPV, negative predictive value. Combined sensitivity and specificity reported for assays which detect multiple immunoglobulins. Information accessible at: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations>, <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance>.

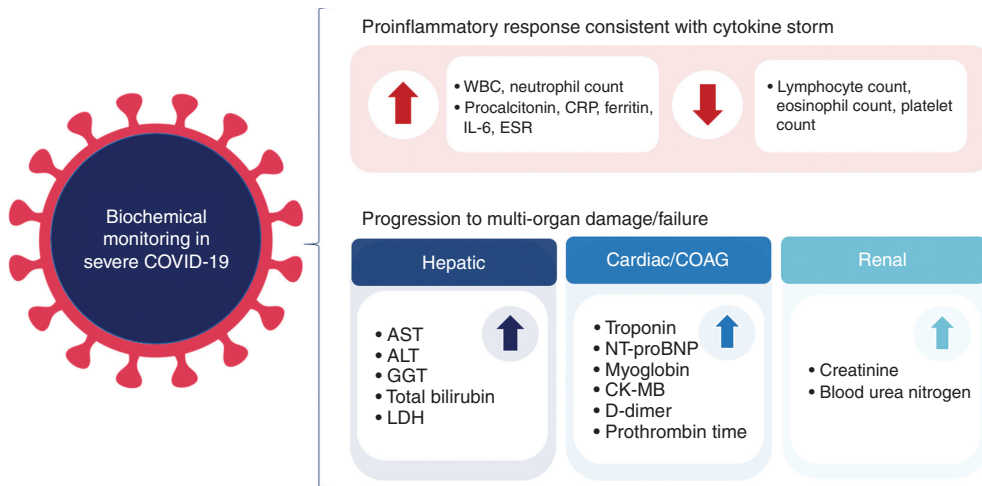


Figure 2: Biochemical and hematological biomarkers of COVID-19 progression and severity.

as well as elevated neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have prognostic potential. Elevation in the D-dimer coagulation parameter has also been consistently reported and associated with worsening disease and a higher risk of developing a wide spectrum of thromboembolic events in COVID-19 patients, including *in situ* pulmonary micro-thrombosis, deep vein thrombosis, overt pulmonary embolism, and even disseminated intravascular coagulation (DIC) [67, 71–73].

Overall, these data confirm that severe cases of COVID-19 are characterized by a massive proinflammatory response or cytokine storm that is estimated to progress to multiple organ damage and failure (i.e. MOF) in severe cases. Biochemical monitoring of COVID-19 patients will thus involve assessing the inflammatory profile, as well as early recognition of cardiac, renal, and hepatic injury through routine laboratory testing.

Cardiac biomarkers

Accumulating clinical data suggest prominent cardiovascular dysfunction in severe COVID-19 patients [74, 75]. Initial reports in China found that 12% of all patients and 31% of intensive care unit (ICU) patients presented with acute myocardial injury, as determined by increased cardiac troponin I levels [76]. Notably, patients with higher cardiac troponin values were more likely to be admitted to intensive care [76, 77] and showed higher in-hospital mortality [66, 78–81]. Another case study series of 187 confirmed COVID-19 patients reported that 27.8% of patients had myocardial injury, which resulted in cardiac dysfunction and arrhythmias [78]. From these clinical data, it is clear that monitoring cardiac biomarkers such as cardiac troponin and

natriuretic peptides throughout disease progression will be essential for appropriate patient risk stratification. The American College of Cardiology (ACC) recently published a statement on the role of cardiovascular biomarker monitoring in patients with COVID-19. They stated that “*clinicians are advised to only measure troponin if the diagnosis of acute myocardial infarction is being considered on clinical grounds*” [82]. This clinical advice is likely to avoid unwarranted diagnostic testing in COVID-19 patients and minimize downstream consultation and procedures, including bedside echocardiography and angiography [83]. However, many groups have rebutted this opinion, emphasizing that cardiac troponin should not be solely considered a binary test for diagnosing myocardial infarction, but a useful prognostic indicator of both ischemic and non-ischemic causes of cardiac dysfunction that can be extremely helpful in patient triage and proper treatment selection [83]. Suspected mechanisms for cardiovascular complications of COVID-19 include: (a) viral myocarditis, (b) direct myocardial injury, (c) cytokine-driven myocardial damage, (d) microangiopathy, and (e) exacerbation of coronary artery disease [75, 84]. Currently, none of these proposed mechanisms have been described as the main contributor to the cardiovascular complications observed in severe COVID-19. Further clinical studies will be important to pinpoint the main driver of myocardial damage for treatment purposes and for guiding laboratory assessment.

Hepatic biomarkers

Recent evidence suggests that liver dysfunction may also be commonplace in severe COVID-19 patients. Several large-scale hospital studies have reported elevations in

select liver enzymes of both hepatic and mixed type, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl-transferase (GGT) [76, 77, 85–90]. A recent study by Cai and colleagues completed a retrospective chart review of clinical and laboratory findings from 417 patients with laboratory-confirmed COVID-19 [89]. Of the 417 patients, 76.3% were reported to have abnormal liver marker results and 21.5% were reported to have liver injury during hospitalization [89]. Interestingly, while both GGT and alkaline phosphatase (ALP) are considered cholangiocyte-related enzymes, only GGT has been implicated in severe COVID-19, thus pinpointing that the damage is likely drug-induced rather than bile duct injury [89]. While it is clear that liver harm is associated with a higher likelihood of developing severe COVID-19, there is much debate regarding the cause of abnormalities in liver injury tests. Potential clinical mechanisms behind liver dysfunction in COVID-19 include: (a) immune-mediated damage due to the severe inflammatory response following infection, (b) direct cytotoxicity due to active viral replication in biliary epithelial cells which express ACE2, (c) hypoxic hepatitis due to anoxia, and (d) drug-induced liver injury [91–93]. As the contribution of underlying hepatic conditions and the clinical mechanism behind COVID-19-related liver dysfunction remains unclear, it is nevertheless evident that liver function tests should be ordered routinely to assess relative hepatic injury in COVID-19 patients within the clinical context, particularly for those receiving antiviral treatment.

Renal biomarkers

Previous data from the SARS epidemic in 2002–2003 reported that 6.7% of patients developed acute renal impairment, and that mortality of SARS patients with acute kidney injury (AKI) was as high as 91.7% [94]. Given the previously emphasized homology between the causative viruses, the evaluation of kidney dysfunction in COVID-19 appears to be quite important. Available data suggest that the prevalence of AKI is relatively low, but variable in COVID-19, ranging between 0.5 and 19.1% in various studies [76, 77, 79, 85, 87, 95, 96]. Both blood urea nitrogen and serum creatinine are known to be routine markers of kidney injury [96]. Indeed, the present method for detecting AKI is mainly based on acute changes in serum creatinine, whilst the frequency of serum creatinine testing has a substantial impact on detection rate [95, 97]. In a recent study by Cheng et al., patients with elevated baseline serum creatinine were more likely to be admitted to the ICU and to undergo

mechanical ventilation [95]. These findings suggest that early identification and treatment of kidney deterioration, including adequate hemodynamic support and limiting nephrotoxic drugs, may be vital in COVID-19 care and increased frequency of creatinine testing or other kidney markers may be warranted. Serum and urinary albumin and total protein could also be useful as prognostic indicators in COVID-19. Similar to liver dysfunction, the pathophysiological mechanisms behind renal dysfunction in COVID-19 patients are unknown and likely multifactorial. Potential mechanisms include: (a) intrarenal inflammation and damage due to cytokine inflammatory response to infection, (b) direct cytopathic effects on kidney tissue, and (c) organ cross-talk (e.g. cardiomyopathy and acute viral myocarditis can contribute to renal vein congestion, hypotension, and renal hypoperfusion) [98].

Through these initial studies, it is evident that COVID-19 disease severity is associated with a strong inflammatory response leading to multi-organ failure. The clinical laboratory can play a critical role in assessing not only inflammatory markers but also closely monitoring indicators of potential organ failure together with typical measures in critical care (i.e. blood gas, electrolytes, etc.).

Considerations for pediatrics

In comparison to adults, the incidence of confirmed pediatric COVID-19 cases appears much lower. Additionally, the reported clinical manifestations of COVID-19 in children are mild, resulting in low severity and very low mortality rates [99, 100]. However, it is clear that children are equally vulnerable to COVID-19 exposure and could be potential carriers, likely resulting in underestimated case reports. The characterization of pediatric response to COVID-19 is incredibly important, particularly given their undeveloped immune system. In a meta-analysis by Lippi et al, 12 articles totaling 66 pediatric patients were evaluated. Their findings reported that a consistent profile of laboratory abnormalities has yet to be observed in COVID-19. However, inflammatory parameters such as lymphocyte count and CRP along with procalcitonin could serve as important prognostic indicators [101]. It is currently unknown why children develop such mild-to-moderate disease with varying laboratory profiles. Some estimated contributors include: limited risk of exposure, fewer comorbidities, and differing immune response with stronger innate and weaker adaptive response in comparison to adults (e.g. immature immunocompetence due to

lower “priming” with other coronaviruses may explain the very low frequency of abnormal immune response against SARS-CoV-2 in childhood) [102]. As the number of global pediatric cases increases, pediatric laboratory profiles throughout the COVID-19 disease course will become clearer.

Conclusions

Laboratory medicine remains at the very core of the diagnostic reasoning and managed care of virtually all human pathologies [103], and COVID-19 is not an exception [104]. The essential role of the clinical laboratory has never been more visible than in today’s crisis. Clinical laboratory medicine is a crucial pillar in our response to COVID-19 by developing molecular and serology assays for diagnosing acute or past infection, and by evaluating the accuracy and clinical utility of tests rapidly developed in an international emergency situation. Clinical laboratories are already greatly contributing to the management and risk stratification of patients through biochemical monitoring of biomarkers of inflammation and MOF. All in all, it is undisputable that the value of laboratory medicine in healthcare has been highlighted in this pandemic and cannot be understated.

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