Journal Pre-proof

Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays

Antonio La Marca , Martina Capuzzo , Tiziana Paglia , Laura Roli , Tommaso Trenti , Scott M Nelson

PII: S1472-6483(20)30318-7
DOI: https://doi.org/10.1016/j.rbmo.2020.06.001
Reference: RBMO 2441

To appear in: *Reproductive BioMedicine Online*

Received date: 2 May 2020
Revised date: 23 May 2020
Accepted date: 2 June 2020

Please cite this article as: Antonio La Marca , Martina Capuzzo , Tiziana Paglia , Laura Roli , Tommaso Trenti , Scott M Nelson , Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays, *Reproductive BioMedicine Online* (2020), doi: https://doi.org/10.1016/j.rbmo.2020.06.001

Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays

Antonio La Marca, M.D., Ph. D.[a], Martina Capuzzo, M.D.[a], Tiziana Paglia, M.D.[b], Laura Roli M.Sc.[c], Tommaso Trenti, M.D.[c], Scott M Nelson, M.D., Ph.D.[d,e,f]

[a]Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Modena, Italy

[b]Department of Anesthesiology, Hesperia Hospital, Modena Italy

[c]Department of Laboratory Medicine and Pathology, Azieda USL Modena, Italy

[d]School of Medicine, University of Glasgow, United Kingdom

[e]NIHR Bristol Biomedical Research Centre, Bristol, United Kingdom;

[f]The Fertility Partnership, Oxford, United Kingdom

Word Count: 9810 (including abstract and references)

Tables:1

Figures: 1

Correspondence: Prof Antonio La Marca, Obstetrics & Gynecology, University Hospital Modena. Email antlamarca@libero.it; antonio.lamarca@unimore.it

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its associated Coronavirus disease 2019 (COVID-19) pandemic has demanded rapid upscaling of in-vitro diagnostic assays to enable mass screening and testing of high-risk groups, and simultaneous ascertainment of robust data on past SARS-CoV-2 exposure at an individual and population level. To meet the exponential demand in testing, there has been an accelerated development of both molecular and serological assays across a plethora of platforms. In the present review, we discuss the current literature on these modalities including the nucleic acid amplification tests, direct viral antigen tests and the rapidly expanding laboratory based and point of care serological tests. This suite of complementary tests will inform crucial decisions by healthcare providers and policy makers and understanding their strengths and limitations will be critical to their judicious application for the development of algorithmic approaches to treatment and public health strategies.

Key Words: SARS-CoV-2, COVID-19, diagnostic test, serology, antibody testing

Introduction

In December 2019, an outbreak of an unexplained pneumonia originated from the city of Wuhan, Hubei Province, China (Huang et al., 2020; Guan et al., 2020). After the initial outbreak, a novel coronavirus (SARS-CoV-2) was quickly identified as the etiological agent, and the associated disease defined as COVID-19 (named as an acronym from CO-rona VI-rus D-isease, where 19 stands for the year the virus was firstly detected). The exponential growth of affected individuals led the World Health Organization (WHO) declaring a global pandemic on the March 11, 2020 (Huang et al., 2020), with 3,002,303 confirmed cases and 208,131 deaths worldwide as of the April 27, 2020, with many more anticipated. The utilization of direct molecular diagnostic testing based on sequencing of SARS-CoV-2, has been critical in identifying infected individuals. However, as lock down measures have begun to bite, there has been a race to develop and approve tests with a different purpose, to assess not current viral infection but rather immunity to severe SARS-CoV-2 to facilitate a return to work. However, antibody testing may also be relevant in our critical evaluation of the disease including: i) understanding the kinetics of the immune response to infection ii) understanding the immune response relative to disease severity and timeline iii) understanding whether cross-reactivity with other coronaviruses leads to cross-protection, iv) clarifying whether infection protects from future infection and how long will immunity last and v) what are the correlates of protection that can guide public health measures. In addition to these critical questions, immediate clinical applications would include i) diagnosis and triage of patients who seek medical attention in the later phases of the disease, ii) contact tracing; iii) stratifying workforces and patients if immunity shown to be lasting and iv) sero-epidemiological studies to understand the extent of COVID-19 spread.

An understanding of the application and diagnostic performance of the different testing approaches for SARS-Cov-2 is essential in the fight against this pandemic. In our own field, these tests are believed by many to be one of the milestones for the recommencement of clinical activity. The

recent ESHRE (www.eshre.eu/Home/COVID19WG) position statement highlighted the current lack of understanding in the field of in-vitro diagnostic assays and in particular serological testing, and the ASRM (www.asrm.org/news-and-publications/covid-19) have called for healthcare providers to be aware of the limitations of these tests. The purpose of this review was to provide an overview of current diagnostic approaches for SARS-CoV-2 and in particular highlight the issues with serological testing with the objective of providing a clear guide to clinicians on the assays currently available.

Methods

A literature search was carried out for studies that focused on the diagnostic and serological testing for SARS-CoV-2, using the keywords coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and COVID-19. PubMed, Google Scholar and Embase databases were searched without language restrictions from inception through to April 16, 2020 and updated on May 15, 2020. Given the rapidly developing field and rapid dissemination of scientific findings with respect to COVID-19 the preprint servers for both health sciences (medRxiv) and biology (bioRxiv) databases were also performed. Additional journal articles were identified from the bibliographies of included studies. For the main objective of this review, all original studies reporting on the sensitivity and/or specificity of antibodies against SARS-CoV-2 were included in the analysis. More than 20,000 articles have been published on SARS-CoV-2, of which 4,182 articles were related to coronavirus and antibodies or serology. After screening of title and abstract, 887 full text studies were retrieved with 66 studies meeting the inclusion criteria and reporting data on test sensitivity and specificity, as summarized in Table 1.

Coronaviral genome and structure

Coronaviruses (CoV) belong to the subfamily Coronavirinae in the family of Coronaviridae of the order Nidovirales. In this subfamily four genera are included: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. The genome of the virus is a single-stranded positive-sense RNA (+ssRNA) (~30 kb) with 5′-cap structure and 3′-poly-A tail. The genome and subgenomes of a typical coronavirus may present six open reading frames (ORFs) or even more. The first ORFs (ORF1a/b), encompass approximately 66% of the whole genome and encode 16 nonstructural proteins (nsp1-16), which are mainly involved in replication of CoVs. Other ORFs encompassing one-third of the genome near the 3′- terminus encode the main structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (Chen et al., 2020a).

The different Coronaviruses exhibit 54% identity of the whole RNA, with 58% identity on the nonstructural proteins-coding region and 43% identity on the structural protein-coding region. Sequence analysis shows that the new coronavirus incorporates the typical genome structure of CoV and belongs to the cluster of betac-CoV that includes Bat- SARS-like (SL)-ZC45, Bat-SL ZXC21, SARS-CoV, and MERS-CoV. Based on the phylogenetic tree of CoVs, 2019-nCoV is more closely related to bat-SL-CoV ZC45 and bat-SL-CoV ZXC21 and more distantly related to SARS-CoV (Chen et al., 2020a)

Four principal structural proteins are essential for virion assembly and its associated infective capacity. Homotrimers of S proteins make up the spikes on the viral surface and they are responsible for attachment to receptors on the host cells. The M protein has three transmembrane domains and it shapes the virions, promotes membrane curvature, and covers the nucleocapsid. The E protein participates in virus assembly and release and is involved in viral pathogenesis. The N protein presents two domains, both of which can bind virus RNA genome via different mechanisms.

The N protein binds to nsp3 protein to help tether the genome to replication-transcription complex and package the encapsidated genome into virions. N protein is also an antagonist of interferon and viral encoded repressor of RNA interference, which may be beneficial for the viral replication.

Diagnostic tests for the SARS-CoV-2

The database held by the Foundation for Innovative New Diagnostics, which is the WHO Collaborating Centre for Laboratory Strengthening and Diagnostic Technology Evaluation, on the 22 May 2019 contained 560 SARS-CoV-2 laboratory tests for the diagnosis of COVID-19. This comprises 273 molecular assays and 287 immunoassays. Excluding those intended for research use only, 152 of these are molecular assays and 211 immunoassays are CE-IVD marked. There are principally two types of tests available for COVID19; viral tests and antibody tests. The viral tests are direct tests as they are designed to detect the virus and therefore reflect current infection. In contrast, the antibody tests are indirect tests, as they do not detect the virus, but rather ascertain established seroconversion to previous infection, or early seroconversion to ongoing infection.

Direct tests

The recommended test for SARS-CoV-2 infection diagnosis is by detecting the viral RNA with nucleic acid amplification tests (NAAT), such as RT-PCR (www.ecdc.europa.eu). In areas with widespread community transmission of SARS-CoV-2 and when laboratory resources are limited, detection by RT-PCR of a single discriminatory target is considered sufficient. There are however, still specific technical considerations for laboratory testing, including specimen collection (variable collection methods), which samples to collect (upper or lower respiratory tract biospecimens, or other samples), time of collection in relation to course of disease and the availability of different

laboratory test methods and kits (not all of which may be standardized or approved by authorities such as the Food and Drug Administration). Then there are the infrastructure considerations, are the approved laboratory facilities and trained manpower available, can the methodology be rapidly scaled up, and how are test results interpreted and false negatives excluded?

These issues have been faced by the whole scientific community, with a collective response to develop guidance. The currently used protocol was developed and optimized for the detection of the novel coronavirus at the Charité University Hospital, in collaboration with several other laboratories in Germany, the Netherlands, China, France, UK and Belgium (Corman et al., 2020). Additionally, the existing protocol was further optimized by the Center for Disease Control (CDC) in the United States through the comprehensive comparison and validation of alternative available kits for nucleic acid extraction and the use of alternative probe and primer sets for efficient SARS-CoV-2 detection in clinical samples (www.cdc.gov/coronavirus). With similar approaches undertaken by other national authorities as they continue to scale up provision for laboratories not using CE marked assays (www.england.nhs.uk/coronavirus/). The importance and variability of specimen collection was initially highlighted on comparison of the positive rates of pharyngeal, nasal, blood, sputum, feces, urine, brochoalveolar lavage fluid and fibrobronchoscope brush biopsy of patients with confirmed COVID-19 (Zou et al., 2019). At present the CDC recommend collecting and testing an upper respiratory specimen, with a nasopharyngeal specimen the preferred choice for swab-based SARS-CoV-2 testing. When collection of a nasopharyngeal swab is not possible, the following are acceptable alternatives; an oropharyngeal specimen, a nasal mid-turbinate (using a flocked tapered swab), an anterior nares (nasal swab) specimen (using a flocked or spun polyester swab) or a nasopharyngeal wash/aspirate or nasal aspirate specimen. For those having invasive procedures lower respiratory tract specimens are also recommended if available. Although detected in other specimens like blood and stools these were generally less reliable than from respiratory specimens.

At present it is recommended that specimens should be collected as soon as possible once a decision has been made to pursue SARS-CoV-2 testing, regardless of the time of symptom onset. The viral load in throat swabs is greatest at the time of viral onset and decrease monotonically thereafter (Zou et al., 2019; To et al., 2020). Analysis of these temporal dynamics suggests that viral shedding may begin 2 to 3 days before the appearance of the first symptoms facilitating pre-symptomatic or asymptomatic transmission (He et al., 2020). CoVs have a number of molecular targets within their positive-sense, single-stranded RNA genome that can be used for RT-PCR assays. The WHO have provided primers for the genes which encode the structural proteins of the viral envelope (E) and the nucleocapsid (N), and for the RNA-dependent RNA polymerase (RdRp), which is a key part of the virus's replication machinery that makes copies of its RNA genome (Corman et al., 2020). However, there has been no demonstration that any one of these three (E, N or RdRP) sequences may offer an advantage for clinical diagnostic testing, with different targets being preferred by different authorities. For example, the Public Health England assay employs two probes against RdRp with one being a Pan Sarbeco-probe which will detect 2019-nCoV, SARS-CoV and bat_SARS-related CoVs while the second probe is specific to 2019-NCoV. Continued refinement of these NAAT assays is ongoing to facilitate their upscaling, while maintaining laboratory safety, a low-cost and high-sensitivity (Won et al., 2020).

Detection of isolated viral antigens

Great efforts have been carried out in order to develop tests for rapid detection of SARS-CoV-2 antigens. Antigen detection tests are designed to directly detect viral particles in biological samples like nasopharyngeal secretions. Several rapid antigen tests have been proposed (Diao et al., 2020) however, the principal concern is the false negative rate due to either a low or variable viral load and the variability in sampling, with the latter having the potential to further compound cases with low viral titres thereby increasing the false negative rate (Tang et al., 2020).

Diao and colleagues (2020) have reported the preliminary results from the utilization of a fluorescence immunochromatographic assay for detecting nucleocapsid protein of SARS-CoV-2 in both nasopharyngeal swab sample and urine from 239 participants, with comparison to NATT testing where the intersection of the amplification curve and diagnostic threshold line (Ct value) was set at either ≤30 or ≤40 (Diao et al., 2020). With a higher viral load in the sample, the prespecified Ct value may be lower, as fewer replication cycles are required to achieve a detectable signal, however, with a low viral load a greater number of replication cycles (higher Ct value) will be required for a detectable signal to be attained. For this assay with a prevalence of 87%, although the positive predictive value was 100%, the negative predictive value was 32% for a Ct ≤40, increasing to 97% for patients with a higher viral load as demonstrated by a Ct≤30. This would suggest that at present this assay would only be useful in excluding those with high viral loads. Whether alternative approaches as previously suggested for influenza viruses in children including the utilization of colloidal gold-labeled IgGs as the detection reagent (Li et al., 2020), to increase the sensitivity of rapid antigen tests for respiratory viruses is feasible is still under consideration, with monoclonal antibodies specifically against SARS-CoV-2 under development. Further validation of these technique and similar approaches in larger populations including asymptomatic cases is warranted. Consideration of approaches to try to concentrate antigen and amplify the detection phase are however likely to be needed for these methods to have any clinical utility (Loeffelholz et al., 2020).

At present (April 25, 2020), the non-governmental organization FIND (https://www.finddx.org/) have listed four CE-marked rapid SARS-CoV-2 antigen detection tests, which are primarily lateral flow immunochromatographic assays based on the presence of a colloid gold conjugate pad and a membrane strip pre-coated with antibodies specific to SARS-CoV-2 antigens on a test line. If SARS-CoV-2 antigens are present in the specimen withdrawn from a nasopharyngeal swab, a visible band appears on the test line as antibody-antigen-antibody gold conjugate complex forms.

The evaluation of these diagnostic tests has however been limited, and their CE-mark means that they manufacturers state that they conform with the relevant EU legislation, but they may still not be available to purchase. According to IVD Directive 98/79/EC, to affix the CE-mark to COVID-19 diagnostic devices to be used by health professionals, the manufacturer has to specify device performance characteristics and self-declare conformity with the safety and performance requirements listed in the Directive. In contrast, self-tests intended to be used by patients themselves must also be assessed by a third party body (a notified body), which for these tests has yet to happen.

Although direct antigen tests are being registered by several health authorities, the sensitivity of these tests is lower than RT-PCR, with previous antigen detecting ELSIAs developed for SARS_CoV having limits of detection of 50pg/ml (Che et al 2004, Di et al 2005). Furthermore, clarification of their specificity for SARS-CoV-2 is awaited, given the potential for cross-reaction with other human coronaviruses. Despite these limitations, the chief advantages of antigen tests including their rapidity (10-30 mins compared to hours for NAAT testing), ease of interpretation and the limited technical skill and infrastructure required as compared to the NAAT based testing, continue to make them worth pursuing. However, experience with influenza antigen testing, invites caution as these tests may have low sensitivity and specificity, moreover, as noted the false negatives rate will be critical (Tang et al., 2020). Their greatest utility if they come to fruition may be in symptomatic patients when the viral load will be at its greatest to enable accurate triage.

Building an indirect test for SARS-CoV-2: serological testing

In contrast to NATT based testing, where as soon as the sequence is known, a diagnostic test can be built, the diagnostic technology and methodology underlying serological test development is quite different, with a substantially longer timeline to obtain a robust product which is suitable for routine deployment. The principal difference is that antibody tests require identification of distinct proteins

that form the viral coat, with elucidation of which proteins are most divergent from previous coronavirus proteins; then identification of specific antibodies to these proteins that are part of the acquired immune response to viral exposure, and finally testing to ensure that there is limited cross-reactivity with antibodies developed to other historical coronaviruses.

With the previous two coronaviruses a variety of assays encompassing different methodologies were developed including ELISA, chemiluminescence, western blot, protein microarray, and immunofluorescence platforms. With only ELISA and chemiluminescence deemed suitable for clinical application because of costs, time-to-results, relative simplicity and ability to scale to very large throughput. It is these platforms which are once again being examined for detection of antibodies to SARS-CoV-2.

Appraisal of test performance

Appropriate thresholds for sensitivity and specificity of an antibody test depend on its purpose and must be considered prior to implementation. For diagnosis in symptomatic patients, high sensitivity is required (generally ≥ 90%). In this context, a slight reduction in specificity may be acceptable as some false positives may be tolerated, provided other potential diagnoses are considered and acceptance that over-diagnosis may result in unnecessary interventions which for SARS-CoV-2 may include quarantining. However, if antibody tests were deployed as an individual-level approach to inform release from social isolation and return to normal activities, then high specificity is essential, as false-positive results return non-immune individuals to risk of exposure. It is with these purposes in mind that the UK Medicines and Healthcare products Regulatory Agency set a minimum 98% specificity threshold for lateral flow immunoassays (LFIAs). This is particularly challenging, particularly given the scale of validation study required for a suitable candidate LFIA as to demonstrate a high specificity if the true underlying value was 98%, 1000 negative controls would be required to estimate the specificity of an assay to +/-1% with approximately 90% power.

As part of the evaluation of test performance the influence of population prevalence also needs to be considered, acknowledging that at present this is rapidly changing (Brenner and Gefeller 1997). This can be considered as the proportion of all positive tests that are wrong, as well as the number of incorrect positive tests per 1000 people tested. For example a point of care test with 70% sensitivity and 98% specificity, the proportion of positive tests that are wrong is 35% at 5% population seroprevalence (19 false-positives/1000 tested), 13% at 20% seroprevalence (16 false-positives/1000) and 3% at 50% seroprevalence (10 false-positives/1000).

According to available data, seropositivity prevalence is still low. The prevalence of antibodies to SARS-CoV-2, among a high risk category such as healthcare personnel is 5.9% in Utah (Masden et al., 2020), 5.4% in Lyon, France (Solodky et al., 2020), 17.3% in Trieste (Comar et al., 2020), 5.25% in Padua (Tosato et al. 2020), 1.5% in Bari, Italy (Paradiso et al., 2020), 1.6% in Germany (Korth et al., 2020) and 2.6% in Barcelona, Spain (Tuaillon et al., 2020). In the general population it has been reported as being 0.13% in Rio Grand do Sul, Brasil (Silveira et al., 2020), 1.5% in Santa Clara, California (Benavid et al. 2020), 1.79 % in Idaho (Bryan et al., 2020) and 7.1% in Atlanta, USA (Zou et al., 2020), 1.2% in Edinburgh, Scotland (Thompson et al., 2020), 3% in Paris, France (Grzelak et al., 2020), 1.7% in Denmark (Erikstrup et al., 2020) and 3.3% in Kobe, Japan (Doi et al., 2020), 9.6% in Whuan, China (Wu et al., 2020) and 21% in Guilan, Iran (Shakiba et al., 2020). Large scale seroprevalence studies are ongoing but understanding the background rates are essential for accurate interpretation of diagnostic tests.

The potential risk of a test providing false reassurance and release from being sheltered for non-immune individuals, can therefore widely based on the underlying seroprevalence and this still assumes antibody-positivity as a correlate of protective immunity, which may be incorrect.

Dynamics of seroconversion

Understanding viral and host interactions during acute and convalescent phases are critical to be able to understand both the timing of initial seroconversion after exposure to SARS-CoV-2, and the subsequent duration of antibodies. However, at present the studies regarding seroconversion are being developed in parallel to the assays, limiting some conclusions. The data does suggest that seroconversion after exposure to SARS-CoV-2 is very similar to other acute viral infections, with IgG concentration beginning to rise as IgM levels reach a plateau (Figure 1). However, observations that IgM and IgA growth is relatively slow related to other respiratory viruses, have been suggested to contribute to the heterogeneous pathogenicity of SARS-CoV-2 in COVID-19 patients (Zhao et al., 2019).

The most comprehensive study to date of seroconversion assessed 173 patients affected by COVID-19 utilizing an assay developed to detect antibodies against the receptor binding domain (RBD) of the spike protein of SARS-CoV-2 (Zhao et al., 2019). The median seroconversion time of total Ab, IgM and IgG antibodies was 11, 12 and 14 days respectively (Zhao et al., 2019). The respective seroconversion rates for total Ab, IgM and IgG were 93.1%, 82.7% and 64.7% (Zhao et al., 2019), with the cumulative seroconversion curve suggesting that the rate for total Ab and IgM reached 100% 30 days after the onset. These studies have also highlighted the temporal nature of testing. As despite all patients being subsequently confirmed as COVID-19 positive, in the early phase of illness (within 7-day since onset), the NATT test only exhibited 66.7% sensitivity with the antibody assays even lower with a positive rate of 38.3% (Zhao et al., 2020). However, the sensitivity of Ab overtook that of RNA test since day 8 after symptom onset and reached over 90% across day 12 after onset. Among samples from patients in later phase (day 15-39 since onset), the sensitivities of total Ab, IgM and IgG were 100.0%, 94.3% and 79.8%, respectively. In contrast, RNA was only detectable in 45.5% of samples of day 15-39. In a separate small series of nine cases, seroconversion was occurred after 7 days in 50% of patients (14 days in all) but was not followed by a rapid decline in viral load (Wolfel et al., 2020). Analysis of 285 patients would further support

IgG seroconversion within 19 days after symptom onset (Long et al 2020). Collectively this data suggests that there is a role for both tests depending on where the patient is on their infection journey, with the combined use of NATT and Ab tests markedly improving the sensitivity of a pathogenic-diagnosis for COVID-19 patients in different phases.

With respect to antibody titres and disease severity, critically ill hospitalized patients have been reported to exhibit significantly higher Ab title values than non-critical cases in some studies (Zhao et al., 2019; Long et al., 2020) but not all studies. In previous epidemics SARS-CoV and the MERS-CoV, antibody titres were positively associated with disease severity (Okba et al., 2019; Choe et al., 2017). In a limited case series (n=57 confirmed SARS-CoV-2 cases), six patients with detectable viral RNA in the blood, were at increased risk of severe disease progression as compared to those with low titres, but unfortunately, the authors did not measure antibody titres (Chen et al., 2020b). Clarification of whether even in previously healthy individuals a high viral titre, and / or high antibody titer can predict disease severity and likely progression is awaited.

Diagnostic performance of the immunoassays

Our extensive search identified 25 peer-reviewed articles and 26 pre-print studies reporting on the sensitivity and specificity of immunoassays for COVID-19 with a sample size ranging from 16 to 6001 subjects (Table 1). Most studies were conducted in China, with only a few coming from western countries. The overall sensitivity ranged from 0% to 100% and the specificity from 78% to 100%, with performance highly time sensitive reflecting the dynamics of seroconversion. In general, most assays performed better shortly after initial symptom resolution, accepting the very limited time frames evaluated for all studies to date. In an evaluation of nine commercially available SARS-CoV-2 immunoassays the sensitivities varied the duration of disease: early phase, 7 to 13 days after the onset of disease symptoms (sensitivities ranged from 40 to 86%); middle phase, 14 to 20 days after the onset of disease symptoms (sensitivities ranged from 67 to 100%); and late phase,

≥21 days after the onset of disease symptoms (sensitivities ranged from 78 to 89%) (Lassauniere et al., 2020).

The range of assays being released is extensive, with apparently very limited validation. Gonzalez and colleagues reviewed four web databases for SARS-CoV-2 immunoassay for, and by the April 4, 2020, there was already 226 immunoassays from 20 different countries. The technical data sheet was available online in only 22% of tests and despite 23 claiming regulatory certification only four had Pubmed listed papers (Gonzalez et al., 2020). Despite wide claims on sensitivity and specificity, practically at present it is almost impossible to conclude which antibody test would be the one to use. A pragmatic choice would be to use an automated immunoassay that is scaleable, from a well-known established manufacturer, with a complete and clear technical data sheet, which has received regulatory certification issued by the health authority and been validated independently.

In accordance with this, the most recent novel assays utilize fully automated chemiluminescence immunoassays (CLIAs) implemented on high throughput laboratory instrumentation. These systems include the MAGLUMI™ 2000 Plus 2019-nCov IgM and IgG assays (Snibe, Shenzhen, China), which has been independently validated in accordance with the Clinical and Laboratory Standards InstituteEP15-A3 guideline (Padoan et al. 2020) and the CE-marked Euroimmun Anti-SARS-CoV-2 IgA and IgG assays, with others including Beckman Coulter for their Access platform and Roche Diagnostics for their Elecsys platform under development. The Euroimmun assay however in independent validation exhibited some cross reactivity in both ELISAs with serum samples from the two seasonal coronavirus patients (HCoV-OC43) that had previously cross-reacted with the MERS-CoV S1 IgG ELISA (Okba et al., 2019). On comparison of their respective performances on 131 known cases, there was only concordance for the IgG assays of 88% (kappa statistics, 0.47; 95% CI, 0.26–0.68). Despite being different immunoglobulin classes, an analogous analysis

between MAGLUMI 2019-nCoV IgM positive/negative vs. Euroimmun Anti-SARS-CoV-2 IgA positive/negative results yielded an overall concordance of 90% (kappa statistics, 0.39; 95% CI, 0.14–0.65). The IgG assays also exhibited different concordance during the early phases of symptom onset, with concordance improved 10-21 days after symptom onset. Further studies with longer timelines and known cases with a range of symptoms will help confirm alignment of these assays. Inevitably we anticipate an enormous number of studies comparing the available assays, with the advantages and disadvantages of the respective assays discussed at length.

Rapid serological tests

Point of care (POC) immunoassays have also been developed for the rapid detection of SARS-CoV-2 antibodies (IgG and IgM). The primary advantage of these tests, like an at home pregnancy test, is to obtain a diagnosis without sending samples to centralized laboratories, thereby enabling communities without the necessary laboratory infrastructure to detect SARS-CoV-2 exposed subjects, use only finger prick testing rather than formal blood draws thereby reducing training requirements and enable clinicians to have a validated test at the bedside. As these devices are cheap to manufacture, store and distribute, provided that a positive antibody test was confirmed to be an accurate surrogate for immunity to infection they would also be able inform decision making. This would be particularly the case as secure confirmation of antibody status would reduce anxiety, provide confidence to allow individuals to relax social distancing measures, and guide policy-makers in the staged release of population lock-down, potentially in tandem with digital approaches to contact tracing.

The rapid point-of-care immunoassays are generally lateral flow immunoassays (LFIA) (Li et al., 2020). In lateral flow assays, a membrane strip is coated with two lines: gold nanoparticle-antibody conjugates are in one line and bind antibodies in the other. The blood sample from the patient is put on the membrane, and the proteins draw through the membrane strip by capillarity. As it passes the

first line, the antigen binds to the gold nanoparticle-antibody conjugate, and the complex flows together across the membrane. Generally, the rapid assays have a low diagnostic performance when compared to ELISA assays and this is explained not only by the well-known technical differences between the two methodologies but also because of possible low antibody concentrations that may further contribute to the false negatives observed with the rapid tests.

At present, 11 peer-reviewed articles and 8 pre-print studies have reported on the diagnostic performance of the rapid assays, these are summarised in Table 1. In the published studies sensitivity and specificity ranged from 9 to 88.6% and from 88.9 to 91.7%, respectively (Table 1), while in the pre-print articles sensitivity and specificity ranged from 30 to 98.8% and from 89 to 100%, respectively. Of note the sensitivity of these tests performed in non-Chinese countries were substantially lower than those reported for studies conducted in China. Extensive evaluation of manufacturers claims on the performance of these tests and optimal timing will be required before they are suitable for widespread routine clinical use. For example, the performance of VivaDiag COVID-19 IgM/IgG Rapid Test was evaluated in 30 cases 7 days (Corman et al., 2020; Tang et al., 2020) after confirmed NATT testing and despite this 5 (16.7%) were negative for both IgG and IgM (Cassaniti et al., 2020). Furthermore, in evaluation of 50 acute patients presenting in the emergency room, the sensitivity of the VivaDiag COVID-19 IgM/IgG Rapid Test was 18.4%, specificity was 91.7%, while NPV was 26.2%, and PPV was 87.5% (Cassaniti et al., 2020). The same VivaDiag test was evaluated in 525 health care workers in Italy with only six testing positive, none were positive by NATT testing or symptomatic and only three had a confirmed positive result on the MAGLUMI chemiluminescence IgG assay (Paradiso et al., 2020b). Evaluation of six POC tests in a mix of 110 cases of COVID-19, other coronavirus, other viruses and negative controls revealed sensitivities ranging from 80 to 93% and negative predictive values of 74 to 92% (Lassauniere et al., 2020). In keeping with other studies, the diagnostic performance of these tests reflected the duration of the illness with the worst performance observed in the first two weeks after symptom

onset (Lassauniere et al., 2020). Lastly formal evaluation of nine commercially available LFIAs in a case control mix of 182 samples revealed sensitives of 55 to 70% (National COVID Testing Scientific Advisory Panel, 2020).

For all studies to date, sample size has been limited, with further testing across a large diverse population from a range of geographical locations and ethnic groups required, with inclusion of children and individuals with autoimmune disease and immunosuppression. With extensive evaluation it is likely that technical performance may deteriorate. At present evaluation of the current LFIA devices suggest that although they may provide some information for population-level surveys, their performance is inadequate for most individual patient applications.

Clinical interpretation of the COVID19 tests

The interpretation of a test for SARS-CoV-2, will depend on a combination of the accuracy of the test and the estimated risk of COVID19 prior to performing the test (Watson et al BMJ 2020). A positive direct antigen test and specifically the nucleic acid amplification tests are strongly suggestive of current infection due to its high specificity but moderate sensitivity, and the patient can be reassured that you are confident that they have COVID19 and should managed in accordance with local policies regarding positive cases. In contrast, negative tests need to be interpreted with caution, and a single negative SARS-CoV-2 test in a patient with strongly suggestive symptoms should not be relied upon to exclude COVID19. In this situation, it would still be safer for the patient to be treated as a positive and local policies regarding retesting and isolation be followed. For the serological tests, the clinical implication of seroconversion with respect to future immunity continue to be elucidated, but similar principles for evaluating the test result in the clinical context and history of previous infection or exposure is critical, particularly as a false positive could lead to false reassurance and inappropriate behaviour that may enhance community disease transmission.

Conclusions

At present NATT based methodologies remain the cornerstone of in-vitro diagnostic assays for SARS-CoV-2. There is an urgent need for development of serological assays with high sensitivity for screening and adequate specificity to avoid unnecessary interventions, and confirmation that seropositivity equates to immunity. At present none of the point of care diagnostics for SARS-CoV-2 appear suitable for wide-scale deployment and large prospective studies are urgently needed to clarify their utility. Evaluation of the performance of the potentially scaleable high-throughput immunoassays is ongoing, however, extensive validation across different populations will be required before they can be routinely used to inform critical decision making for clinicians, the public health community and policy-makers.

Author's role

ALM, MC and SMN performed the literature search, the analysis of the studies and wrote the manuscript. TP, LR and TT reviewed, edited and approved the manuscript.

Funding

This work was supported by the National Institute for Health Research Biomedical Centre at the University Hospitals Bristol NHS Foundation Trust and the University of Bristol (SMN). The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health and Social Care, or any other funders mentioned here.

Competing interests

No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. ALM has participated in Advisory Boards and received speakers and consultancy fees from Beckman Coulter, Gedeon Richeter, Ferring, IBSA, Merck, MSD, Roche Diagnostics and Theramex. SMN has participated in Advisory Boards and received speakers and consultancy fees from Access Fertility, Beckman Coulter, Ferring, Finox, Merck, MSD, Roche Diagnostics and The Fertility Partnership. Other Authors declared no conflict of interests.

References

Adams ER, Augustin Y, Byrne RL, Clark DJ, Cocozza M, Cubas-Atienzar AI et al., Rapid development of COVID-19 rapid diagnostics for low resource settings: accelerating delivery through transparency, responsiveness, and open collaboration medRxiv 2020.04.29.20082099; doi: https://doi.org/10.1101/2020.04.29.20082099

Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M,Jiang K, Arunkumar GA, Jurczyszak D, Polanco J, Bermudez-Gonzalez M, Kleiner G,Aydillo T, Miorin L, Fierer DS, Lugo LA, Kojic EM, Stoever J, Liu STH,Cunningham-Rundles C, Felgner PL, Moran T, García-Sastre A, Caplivski D, Cheng AC, Kedzierska K, Vapalahti O, Hepojoki JM, Simon V, Krammer F. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med. 2020. doi:10.1038/s41591-020-0913-5.

Bendavid E, Mulaney B, Sood N, Shah S, Ling E, Bromley-Dulfano R, et al., COVID-19 Antibody Seroprevalence in Santa Clara County, California. medRxiv 2020.04.14.20062463; doi: https://doi.org/10.1101/2020.04.14.20062463

Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, Jerome KR, Mathias PC, Greninger AL. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. J Clin Microbiol. 2020 May 7. pii: JCM.00941-20. doi: 10.1128/JCM.00941-20

Brenner H, and Gefeller O. Variation of sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. Statistics in medicine 1997; 16: 981-991.

Burbelo PD, Francis XR, Morishima C, Rawlings S, Smith D, Das S et al., Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients https://doi.org/10.1101/2020.04.20.20071423

Cai XF, Chen J, Hu JL, Long QX, Deng HJ, Fan K, Liao P, Liu BZ, Wu GC, Chen YK, Li ZJ, Wang K, Zhang XL, Tian WG, Xiang JL, Du HX, Wang J, Hu Y, Tang N, Lin Y, Ren JH, Huang LY, Wei J, Gan CY, Chen YM, Gao QZ, Chen AM, He CL, Wang DX, Hu P, Zhou FC, Huang AL,

Liu P, Wang DQ. A Peptide-based Magnetic Chemiluminescence Enzyme Immunoassay for Serological Diagnosis of Coronavirus Disease 2019 (COVID-19). J Infect Dis. 2020 May 8. pii: jiaa243. doi: 10.1093/infdis/jiaa243.

Cassaniti I, Novazzi F, Giardina F, Salinaro F, Sachs M, Perlini S, Bruno R, Mojoli F, Baldanti F; Members of the San Matteo Pavia COVID-19 Task Force. Performance of VivaDiag COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department. J Med Virol. 2020. doi: 10.1002/jmv.25800

Che, X. Y.; Qiu, L. W.; Pan, Y. X.; Wen, K.; Hao, W.; Zhang, L. Y.; Wang, Y. Di; Liao, Z. Y.; Hua, X.; Cheng, V. C. C.; Yuen, K. Y. Sensitive and Specific Monoclonal Antibody-Based Capture Enzyme Immunoassay for Detection of Nucleocapsid Antigen in Sera from Patients with Severe Acute Respiratory Syndrome. J. Clin. Microbiol. 2004, 42), 2629–2635. https://doi.org/10.1128/JCM.42.6.2629-2635.2004.

Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol. 2020a;92:418-423.

Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, Li L, He R, Tan Y, Deng X, Gao M, Tang G, Zhao L, Wang J, Fan Q, Wen C, Tong Y, Tang Y, Hu F, Li F, Tang X. Detectable 2019-CoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect. 2020b; 9:469-473

Choe PG, Perera RAPM, Park WB, Song KH, Bang JH, Kim ES, et al., MERS-CoV Antibody Responses 1 Year after Symptom Onset, South Korea, 2015. Emerg. Infect. Dis. 2017; 23:1079–1084

Comar M, Brumat M, Concas MP, Argentini G, Bianco A, Bicego L et al. COVID-19 experience: first Italian survey on healthcare staff members from a Mother-Child Research hospital using combined molecular and rapid immunoassays test doi: https://doi.org/10.1101/2020.04.19.20071563

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 doi: 10.2807/1560-7917.ES.2020.25.3.2000045

Demey B, Daher N, François C, Lanoix JP, Duverlie G, Castelain S, Brochot E. Dynamic profile for the detection of anti-SARS-CoV-2 antibodies using four immunochromatographic assays.J Infect. 2020 May 7. pii: S0163-4453(20)30244-9. doi: 10.1016/j.jinf.2020.04.033

Di B, Hao W, Gao Y, Wang M, Wang Y, Di Qiu, et al. Monoclonal Antibody-Based Antigen Capture Enzyme-Linked Immunosorbent Assay Reveals High Sensitivity of the Nucleocapsid Protein in Acute-Phase Sera of Severe Acute Respiratory Syndrome Patients. Clin. Diagn. Lab. Immunol. 2005, 12 (1), 135–140. https://doi.org/10.1128/CDLI.12.1.135-140.2005

Diao B, Wen K, Chen J, Liu Y, Yuan Z, Han C, Chen J, Pan Y, Chen L, Dan Y, Wang J , Chen Y, Deng G, Zhou H, Wu Y. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. medRxiv 2020.03.07.20032524; doi: https://doi.org/10.1101/2020.03.07.20032524

Döhla M, Boesecke C, Schulte B, Diegmann C, Sib E, Richter E, Eschbach-Bludau M, Aldabbagh S, Marx B, Eis-Hübinger AM, Schmithausen RM, Streeck H. Rapid point-of-care testing for SARS-CoV-2 in a community screening setting shows low sensitivity. Public Health. 2020 ;182:170-172.

Doi A, Iwata K, Kuroda H, Hasuike T, Nasu S, Kanda A, Nagao T, Nishioka H, Tomii K, Morimoto T, Kihara Y. Estimation of seroprevalence of novel coronavirus disease (COVID-19) using preserved serum at an outpatient setting in Kobe, Japan: A cross-sectional study. medRxiv 2020.04.26.20079822; doi: https://doi.org/10.1101/2020.04.26.20079822

Du Z, Zhu F, Guo F, Yang B, Wang T. Detection of antibodies against SARS-CoV-2in patients with COVID-19. J Med Virol. 2020 Apr . doi: 10.1002/jmv.25820.

Erikstrup C, Hother CE, Pdersen OBV, Molbak K, Skov RL, Holm DK et al.Estimation of SARS-CoV-2 infection fatality rate by real-time antibody screening of blood donors medRxiv 2020.04.24.20075291; doi: https://doi.org/10.1101/2020.04.24.20075291

Garcia FP, Tanoira P, Romanyk Cabrera JP, Serrano T, Herruz PG, Gonzalez JC Rapid diagnosis of SARS-CoV-2 infection by detecting IgG and IgM antibodies with an immunochromatographic device: a prospective single-center study MedRxiv /doi.org/10.1101/2020.04.11.20062158

González JM , Shelton WJ, Díaz-Vallejo M, Rodriguez-Castellanos VE, Zuluaga JDH, Chamorro DF , Arroyo-Ariza D. Immunological assays for SARS-CoV-2: an analysis of available commercial tests to measure antigen and antibodies MedRxiv https://doi.org/10.1101/2020.03.17.20037713

Garcia-Basteiro AL,Moncunill G, Tortajada M, Vidal M, Guinovart C, Jimenez A, Santano R, Sanz S, Mendez S, Llupia A, Aguilar R et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospitalmedRxiv 020.04.27.20082289; doi: https://doi.org/10.1101/2020.04.27.20082289

Grzelak L, Temmam S, Planchais C, Demeret C, Huon C, Guivel F et al. SARS-CoV-2 serological analysis of COVID-19 hospitalized patients, pauci-symptomatic individuals and blood donors. medRxiv 2020.04.21.20068858; doi: https://doi.org/10.1101/2020.04.21.20068858

Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020 . doi: 10.1056/NEJMoa2002032.

Guo L, Ren L, Yang S, Xiao M, Chang, Yang F, Dela Cruz CS, Wang Y, Wu C, Xiao Y, Zhang L, Han L, Dang S, Xu Y, Yang Q, Xu S, Zhu H, Xu Y, Jin Q, Sharma L, Wang L, Wang J. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease(COVID-19). Clin Infect Dis. 2020. doi: 10.1093/cid/ciaa310.

He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al.Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med. 2020 doi: 10.1038/s41591-020-0869-5.

Hoffman T, Nissen K, Krambrich J, Rönnberg B, Akaberi D, Esmaeilzadeh M, Salaneck E, Lindahl J, Lundkvist Å. Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS-CoV-2. Infect Ecol Epidemiol. 2020;10:1754538.

Hou H, Wang T, Zhang B, Luo Y, Mao L, Wang F, Wu S, Sun Z. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. Clin Transl Immunology. 2020 May 6;9(5):e01136. doi: 10.1002/cti2.1136

Hu Q, Cui X, Liu X, Peng B, Jiang J, Wang X et al., The production of antibodies for SARS-CoV-2 and its clinical implicationdoi: https://doi.org/10.1101/2020.04.20.20065953

Huang X, Wei F, Hu L, Wen L, Chen K. Epidemiology and Clinical Characteristics of COVID-19. Arch Iran Med. 2020 ;23:268-271. doi: 10.34172/aim.2020.09. Imai K, Tabata S, Ikeda M, Noguchi S, Kitagawa Y, Matuoka M, Miyoshi K, Tarumoto N, Sakai J, Ito T, Maesaki S, Tamura K, Maeda T. Clinical evaluation of an immunochromatographic IgM/IgG antibody assay and chest computed tomography for the diagnosis of COVID-19. J Clin Virol. 2020;128:104393. doi:10.1016/j.jcv.2020.104393.

Infantino M, Grossi V, Lari B, Bambi R, Perri A, Manneschi M, Terenzi G,Liotti I, Ciotta G, Taddei C, Benucci M, Casprini P, Veneziani F, Fabbri S,Pompetti A, Manfredi M. Diagnostic accuracy of an automated chemiluminescentimmunoassay for anti-SARS-CoV-2 IgM and IgG antibodies: an Italian experience. J Med Virol. 2020. doi: 10.1002/jmv.25932.

Jääskeläinen AJ, Kekäläinen E, Kallio-Kokko H, Mannonen L, Kortela E, Vapalahti O, Kurkela S, Lappalainen M. Evaluation of commercial and automated SARS-CoV-2 IgG and IgA ELISAs using coronavirus disease (COVID-19) patient samples. Euro Surveill. 2020 May;25(18). doi: 10.2807/1560-7917.ES.2020.25.18.2000603.

Jia X, Zhang P, Tian Y, Wang J, Zeng H, Wang J, Liu J, Chen Z, Zhang L, He H, He K, Liu Y. Clinical significance of IgM and IgG test for diagnosis of highly suspected COVID-19 infection. medRxiv 2020.02.28.20029025; doi: https://doi.org/10.1101/2020.02.28.20029025

Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, Wang Y, Cui H, Pan K, Xu A. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. Int J Infect Dis. 2020;94:49-52. doi: 10.1016/j.ijid.2020.03.065.

Korth J, Wilde B, Dolff S, Anastasiou OE, Krawczyk A, Jahn M, Cordes S, Ross B, Esser S, Lindemann M, Kribben A, Dittmer U, Witzke O, Herrmann A. SARS-CoV-2-specific antibody detection in healthcare workers in Germany with direct contact to COVID-19 patients. J Clin Virol. 2020 May 13:104437. doi:10.1016/j.jcv.2020.104437.

Lassaunière R, Frische A, Harboe Z, Nielsen A, Fomsgaard A, Krogfelt K, Jørgensen C. Evaluation of nine commercial SARS-CoV-2 immunoassays. medRxiv doi: 10.1101/2020.04.09.20056325

Lee YL, Liao CH, Liu PY, Cheng CY, Chung MY, Liu CE, Chang SY, Hsueh PR. Dynamics of anti-SARS-Cov-2 IgM and IgG antibodies among COVID-19 patients. J Infect. 2020 Apr 23. pii: S0163-4453(20)30230-9. doi: 0.1016/j.jinf.2020.04.019.

Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, Sun R, Wang Y, Hu B, Chen W, Zhang Y, Wang J, Huang B, Lin Y, Yang J, Cai W, Wang X, Cheng J, Chen Z, Sun K, Pan W, Zhan Z, Chen L, Ye F. 2020. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol 27:25727

Lin D, Liu L, Zhang M, Hu Y, Yang Q, Guo J, Dai Y, Xu Y, Cai Y, Chen X, Huang K, Zhang Z. Evaluations of serological test in the diagnosis of 2019 1 novel coronavirus (SARS-CoV-2) infections during the COVID-19 outbreak. medRxiv 2020.03.27.20045153; doi: https://doi.org/10.1101/2020.03.27.20045153

Lippi G, Salvagno GL, Pegoraro M, Militello V, Caloi C, Peretti A, Gaino S,Bassi A, Bovo C, Lo Cascio G. Assessment of immune response to SARS-CoV-2 withfully automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays.Clin Chem Lab Med. 2020 Apr 16.pii:/j/cclm.ahead-of-print/cclm-2020-0473/cclm-2020-0473.xml.doi:10.1515/cclm-2020-0473.

Liu L, Liu W, Wang S, Zheng S. A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients. medRxiv 2020.03.06.20031856; doi: https://doi.org/10.1101/2020.03.06.20031856

Liu R, Liu X, Han H, Shereehn MA, Niu Z, Li D et al. The comparative superiority of IgM-IgG antibody test to real-time reverse transcriptase PCR detection for SARS-CoV-2 infection diagnosis doi: https://doi.org/10.1101/2020.03.28.20045765

Liu Y, Liu Y, Diao B, Ren F, Wang Y, Ding J, Huang O. Diagnostic Indexes of a Rapid IgG/IgM Combined Antibody Test for SARS-CoV-2. MedRxiv doi: https://doi.org/10.1101/2020.03.26.20044883

Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections – the state of the art. Emerg Microbes Infect. 2020 Dec;9(1):747-756. doi: 10.1080/22221751.2020.1745095.

Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, Liao P, Qiu JF, Lin Y, Cai XF, Wang DQ, Hu Y, Ren JH, Tang N, Xu YY, Yu LH, Mo Z, Gong F, Zhang XL, Tian WG,Hu L, Zhang XX, Xiang JL, Du HX, Liu HW, Lang CH, Luo XH, Wu SB, Cui XP, Zhou Z, Zhu MM, Wang J, Xue CJ, Li XF, Wang L, Li ZJ, Wang K, Niu CC, Yang QJ, Tang XJ,Zhang Y, Liu XM, Li JJ, Zhang DC, Zhang F, Liu P, Yuan J, Li Q, Hu JL, Chen J,Huang AL. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med.2020 Apr 29. doi: 10.1038/s41591-020-0897-1. [Epub ahead of print] PubMed PMID:32350462.

Lou B, Li TD, Zheng SF, Su YY, Li ZY, Liu W, Yu F, Ge SX, Zou QD, Yuan Q, Lin S, Hong CM, Yao XY, Zhang XJ, Wu DH, Zhou GL, Hou WH, Li TT, Zhang YL, Zhang SY, Fan J, Zhang J, Xia NS, Chen Y. Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. medRxiv 2020.03.23.20041707; doi: https://doi.org/10.1101/2020.03.23.20041707

Ma H, Zeng W, He H, Zhao D, Yang Y, Jiang D, et al., COVID-19 diagnosis and study of serum SARS-CoV-2 specific IgA, IgM and IgG by a quantitative and sensitive immunoassay doi: https://doi.org/10.1101/2020.04.17.20064907

Madsen T, Levin N, Niehus K, Law K, Mayer J, Chapman M, Johnson A, Hartsell S.Prevalence of IgG antibodies to SARS-CoV-2 among emergency department employees. Am J Emerg Med. 2020 May 3. pii: S0735-6757(20)30306-5.

Meyer B, Torriani G, Yerly S, Mazza L, Calame A, Arm-Vernez I et al. Validation of a commercially available SARS-CoV-2 serological Immunoassay medRxiv 2020.05.02.20080879; doi: https://doi.org/10.1101/2020.05.02.20080879

Montesinos I, Gruson D, Kabamba B, Dahma H, Van den Wijngaert S, Reza S, Carbone V, Vandenberg O, Gulbis B, Wolff F, Rodriguez-Villalobos H. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. J Clin Virol. 2020 May 5;128:104413. doi:10.1016/j.jcv.2020.104413.

National COVID Testing Scientific Advisory Panel. Evaluation of antibody testing for SARS-Cov-2 using ELISA and lateral flow immunoassay doi: https://doi.org/10.1101/2020.04.15.20066407

Norman M, Gilboa T, Ogata FA, Maley AM, Cohen L, Cay Y et al. Ultra-Sensitive High-Resolution Profiling of Anti-SARS-CoV-2 Antibodies for Detecting Early Seroconversion in COVID-19 Patients medRxiv 2020.04.28.20083691; doi: https://doi.org/10.1101/2020.04.28.20083691

Okba NMA, Raj VS, Widjaja I, GeurtsvanKessel CH, de Bruin E, Chandler FD, et al.,Sensitive and Specific Detection of Low-Level Antibody Responses in Mild Middle East Respiratory Syndrome Coronavirus Infections. Emerg Infect Dis.2019 ;25:1868-1877

Ozturk T, Howell C, Benameur K, Ramonell RP, Cashman K, Pirmohammed S Cross-sectional IgM and IgG profiles in SARS-CoV-2 infection. medRxiv 2020.05.10.20097535; doi: https://doi.org/10.1101/2020.05.10.20097535

Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for 2019-nCov IgM/IgG and antibody kinetics. Clin Chem Lab Med 2020 doi: 10.1515/cclm-2020-0443

Padoan A, Sciacovelli L, Basso D, Negrini D, Zuin S, Cosma C, Faggian D,Matricardi P, Plebani M. IgA-Ab response to spike glycoprotein of SARS-CoV-2 innpatients with COVID-19: A longitudinal study. Clin Chim Acta. 2020b 25;507:164-166.

Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. 2020. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020; 24:30113-4.

Pan Y, Li X, Yang G, Fan J, Tang Y, Zhao J, Long X, Guo S, Zhao Z, Liu Y, Hu H, Xue H, Li Y. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. J Infect. 2020 b. pii: S0163-4453(20)30175-4. doi: 10.1016/j.jinf.2020.03.05

Paradiso AV, De Summa S, Silvestris N, Tommasi S, Tufaro A, De Palma G, Larocca AMV, Chironna M, D'Addabbo V, Raffaele D, Cafagna V, Garrisi V. Rapid serological tests have a role in asymptomatic health workers COVID-19 screening. medRxiv 2020.04.15.20057786; doi: https://doi.org/10.1101/2020.04.15.20057786

Paradiso AV, De Summa S, Loconsole D, Procacci V, Sallustio A, Centrone F, Silvestris N, Cafagna V, De Palma G, Tufaro A, Garrisi V, Chironna M. Clinical meanings of rapid serological assay in patients tested for SARS-Co2 RT-PCR. medRxiv 2020.04.03.20052183; doi: https://doi.org/10.1101/2020.04.03.20052183

Perera RA, Mok CK, Tsang OT, Lv H, Ko RL, Wu NC, Yuan M, Leung WS, Chan JM, Chik TS, Choi CY, Leung K, Chan KH, Chan KC, Li KC, Wu JT, Wilson IA, Monto AS, Poon LL, Peiris M. Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), March 2020. Euro Surveill. 2020 Apr;25(16). doi: 10.2807/1560-7917.ES.2020.25.16.2000421.

Qian C, Zhou M, Cheng F, Lin X, Gong Y, Xie X, et al. Development and Multicenter Performance Evaluation of The First Fully Automated SARS-CoV-2 IgM and IgG Immunoassays medRxiv 2020 doi.org/10.1101/2020.04.16.20067231

Qu J, Wu C, Li X, Zhang G, Jiang Z, Li X, Zhu Q, Liu L. Profile of IgG and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis. 2020 Apr 27. pii: ciaa489. doi: 10.1093/cid/ciaa489.

Rosado J, Cockram C, Merkling H, Demeret C, Meola A, Kerneis S. Serological signatures of SARS-CoV-2 infection: Implications for antibody-based diagnostics medRxiv 2020.05.07.20093963; doi: https://doi.org/10.1101/2020.05.07.20093963

Shakiba M, Nazari S, Mehrabian F, Rezvani S, Ghasempour Z, Heidarzadeh A Seroprevalence of COVID-19 virus infection in Guilan province, Iran. medRxiv 2020.04.26.20079244; doi: https://doi.org/10.1101/2020.04.26.20079244

Shen B, Zheng Y, Zhang X, Zhang W, Wang D, Jin J, Lin R, Zhang Y, Zhu G, Zhu H, Li J, Xu J, Ding X, Chen S, Lu R, He Z, Zhao H, Ying L, Zhang C, Lv D, Chen B,Chen J, Zhu J, Hu B, Hong C, Xu X, Chen J, Liu C, Zhou K, Li J, Zhao G, Shen W,Chen C, Shao C, Shen X, Song J, Wang Z, Meng Y, Wang C, Han J, Chen A, Lu D, Qian B, Chen H, Gao H. Clinical evaluation of a rapid colloidal gold immunochromatography assay for SARS-Cov-2 IgM/IgG. Am J Transl Res. 2020;12:1348-1354.

Silveira M, Barros A, Horta B, Pellanda L, Victora G, Dellagostin O et al., Repeated population-based surveys of antibodies against SARS-CoV-2 in Southern Brazil medRxiv 2020.05.01.20087205; doi: https://doi.org/10.1101/2020.05.01.20087205

Solodky ML, Galvez C, Russias B, Detourbet P, N'Guyen-Bonin V, Herr AL,Zrounba P, Blay JY. Lower detection rates of SARS-COV2 antibodies in cancer patients vs healthcare workers after symptomatic COVID-19. Ann Oncol. 2020 Apr 30. pii: S0923-7534(20)39793-3.

Spicuzza L, Montineri A, Manuele R, Crimi C, Pistorio MP, Campisi R, Vancheri C, Crimi N. Reliability and usefulness of a rapid IgM-IgG antibody test for the diagnosis of SARS-CoV-2 infection: A preliminary report. J Infect. 2020 Apr 23. pii: S0163-4453(20)30231-0. doi: 10.1016/j.jinf.2020.04.022.

Sun B, Feng Y, Mo X, Zheng P, Wang Q, Li P, Peng P, Liu X, Chen Z, Huang H, Zhang F, Luo W, Niu X, Hu P, Wang L, Peng H, Huang Z, Feng L, Li F, Zhang F, Li F, Zhong N, Chen L. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes Infect. 2020;9:940-948. doi: 10.1080/22221751.2020.1762515.

Tang YW, Schmitz JE, Persing DH, Stratton CW The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges. J Clin Microbiol. 2020. pii: JCM.00512-20. doi: 10.1128/JCM.00512-20.

Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, Farnsworth CW. Clinical Performance of Two SARS-CoV-2 Serologic Assays. Clin Chem. 2020 May 13. pii: hvaa120. doi: 10.1093/clinchem/hvaa120. [Epub ahead of print] PubMedPMID: 32402061.

Thompson C, Grayson N, Paton RS, Lourenco J, Penman BS, Lee L, et al., Neutralising antibodies to SARS coronavirus 2 in Scottish blood donors – a pilot study of the value of serology to determine population exposure. medRxiv 2020.04.13.20060467; doi: https://doi.org/10.1101/2020.04.13.20060467

To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study Lancet Infect Dis. 2020 doi: 10.1016/S1473-3099(20)30196

Tosato F, Pelloso M, Gallo N, Giraudo C, Llanaj G, Cosma C et al., 2020 Severe Acute Respiratory Syndrome Coronavirus 2 Serology in Asymptomatic Healthcare Professionals: Preliminary Experience of a Tertiary Italian Academic Center.

MedRxiv 2020.04.27.20073858; doi: https://doi.org/10.1101/2020.04.27.20073858

Tuaillon E. Detection of SARS-CoV-2 antibodies using commercial assays and seroconversion patterns in hospitalized

patientsMedRxiv 2020.05.04.20090027; doi: https://doi.org/10.1101/2020.05.04.20090027

Wang B, Wang L, Kong X, Geng J, Xiao D, Ma C, Jiang XM, Wang PH. Long-term Coexistence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with Antibody Response in Coronavirus Disease 2019 (COVID-19) Patients. medRxiv 2020.04.13.20040980; doi: https://doi.org/10.1101/2020.04.13.20040980

Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. 2020. Detection of SARS-CoV-2 in 386 Different Types of Clinical Specimens. JAMA. 2020 . doi: 10.1001/jama.2020.3786. [Epub ahead of print]

Wang X, Guo X, Xin Q, Chu Y, Li J, Pan Y, Feng Y, Wang Q. Neutralizing Antibodies Responses to SARS-CoV-2 in COVID-19 Inpatients and Convalescent Patients. medRxiv 2020.04.15.20065623; doi: https://doi.org/10.1101/2020.04.15.20065623

Wang Z, Li H, Li J, Yang C, Guo X, Hu Z, Chen Z, Wang S, Liu J. Elevated serum IgM levels indicate poor outcome in patients with coronavirus disease 2019 pneumonia: a retrospective case-control study. medRxiv 2020.03.22.20041285; doi: https://doi.org/10.1101/2020.03.22.20041285

Wajnberg A, Mansour M, Leven E, Bouvier NM, Patel G, Firpo A et al., Humoral immune response and prolonged PCR positivity in a cohort of 1343 SARS-CoV 2 patients in the New York City region MedRxiv 2020.04.30.20085613; doi: https://doi.org/10.1101/2020.04.30.20085613

Wan Y; Li Z, Wank K, Li T, Liao P. Performance verification of detecting COVID-19 specific antibody by using four chemiluminescence immunoassay systems
medRxiv 2020.04.27.20074849; doi: https://doi.org/10.1101/2020.04.27.20074849

Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020 doi: 10.1038/s41586-020-2196-x.

Watson J, Whiting PF, Brush Je. Interpreting a Covid-19 test results. BMJ 2020; 369 doi: https://doi.org/10.1136/bmj.m1808

Won J, Lee S, Park M, Kim TY, Park MG, Choi BY, Kim D, Chang H, Kim VN, Lee CJ. Development of a Laboratory-safe and Low-cost Detection Protocol for SARS-CoV-2 of the Coronavirus Disease 2019 (COVID-19). Exp Neurobiol. 2020 . doi: 10.5607/en20009.

Wu X, Fu B, Chen L, Feng Y. Serological tests facilitate identification of asymptomatic SARS-CoV-2 infection in Wuhan, China. J Med Virol. 2020 Apr 20. doi:10.1002/jmv.25904.

Xiang F, Wang X, He X, Peng Z, Yang B, Zhang J, Zhou Q, Ye H, Ma Y, Li H, Wei X, Cai P, Ma WL. Antibody Detection and Dynamic Characteristics in Patients with COVID-19. Clin Infect Dis. 2020 doi: 10.1093/cid/ciaa461.

Xiang J, Yan M, Li H, Liu T, Lin C, Huang S, Shen C. Evaluation of Enzyme-Linked Immunoassay and Colloidal Gold-Immunochromatographic Assay Kit for Detection of Novel Coronavirus (SARS-Cov-2) Causing an Outbreak of Pneumonia (COVID-19). medRxiv 2020.02.27.20028787; doi: https://doi.org/10.1101/2020.02.27.20028787

Xiao DAT, Gao DC, Zhang DS.Profile of Specific Antibodies to SARS-CoV-2: The First Report. J Infect. 2020 doi: 10.1016/j.jinf.2020.03.012.

Yong G, Yi Y, Tuantuan L, Xiaowu L, Xiuyong L, Ang L, Mingfeng H. Evaluation of the auxiliary diagnosis value of antibodies assays for the detection of novel coronavirus (SARS-Cov-2). medRxiv 2020.03.26.20042044; doi: https://doi.org/10.1101/2020.03.26.20042044

Zeng F, Dai C, Cai P, Wang J, Xu L, Li Jet al., . A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: a possible reason underlying different outcome between gender. medRxiv 2020.03.26.20040709; doi: https://doi.org/10.1101/2020.03.26.20040709

Zhang J, Liu J, Li N, Liu Y, Ye R, Qin X, Zheng R. Serological detection of 2019-nCoV respond to the epidemic: A useful complement to nucleic acid testing. medRxiv 2020.03.04.20030916; doi: https://doi.org/10.1101/2020.03.04.20030916

Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. doi:https://doi.org/10.1093/cid/ciaa344

Zhao R, Li M, Song H, Chen J, Ren W, Feng Y, Gao GF, Song J, Peng Y, Su B, Guo X, Wang Y, Chen J, Li J, Sun H, Bai Z, Cao W, Zhu J, Zhang Q, Sun Y, Sun S, Mao X, Su J, Chen X, He A, Gao W, Jin R, Jiang Y, Sun L. Early detection of SARS-CoV-2 antibodies in COVID-19 patients as a serologic marker of infection. Clin Infect Dis. 2020 May 1. pii: ciaa523. doi: 10.1093/cid/ciaa523

Zhong L, Chuan J, Gong BO, Shuai P, Zhou Y, Zhang Y, et al., Detection of serum IgM and IgG for COVID-19 diagnosis. Sci China Life Sci. 2020. doi:10.1007/s11427-020-1688-9.

Zou J, Bretin A, Gewirtz A Antibodies to SARS/CoV-2 in arbitrarily-selected Atlanta residents medRxiv 2020.05.01.20087478; doi: https://doi.org/10.1101/2020.05.01.20087478

Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. 2020. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N Engl J Med 2020;382:1177-1179

Zhou Q, Zhu D, Yan H, Quan J, Kuang Z, Zhang, W et al., A preliminary study on analytical performance of serological assay for SARS-CoV-2 IgM/IgG and application in clinical practice medRxiv 2020.05.05.20092551; doi: https://doi.org/10.1101/2020.05.05.20092551

Yangchun F. Optimize Clinical Laboratory Diagnosis of COVID-19 from Suspect Cases by Likelihood Ratio of SARS-CoV-2 IgM and IgG antibody medRxiv 2020.04.07.20053660; doi: https://doi.org/10.1101/2020.04.07.20053660

Yong G, Yi Y, Tuantuan L, Xiaowu W, Xiuyong L, Ang L, Mingfeng H. Evaluation of the auxiliary diagnostic value of antibody assays for the detection of novel coronavirus (SARS-CoV-2). J Med Virol. 2020 Apr 22. doi: 10.1002/jmv.25919.

Xiao T, Wang Y, Yuan J, Ye H, Wei L, Wang H et al. Early viral clearance and antibody kinetics of COVID-19 among asymptomatic carriers medRxiv 2020.04.28.20083139; doi: https://doi.org/10.1101/2020.04.28.20083139

Xie J, Ding C, Li J, Wang Y, Guo H, Lu Z, Wang J, Zheng C, Jin T, Gao Y, He H. Characteristics of patients with coronavirus disease (COVID-19) confirmed using an IgM-IgG antibody test. J Med Virol. 2020 Apr 24. doi: 10.1002/jmv.25930.

Antonio La Marca is Professor of Obstetrics and Gynecology at the University of Modena and Reggio Emilia, Italy. His clinical activity covers all fields of reproductive medicine and surgery.
He has published more than 160 peer-reviewed articles; his current h -index is 44, with more than 8,000 citations.

Key Message

Molecular and serological assays for SARS-CoV-2 are being developed and implemented at an exponential rate. This suite of complementary tests will inform crucial decisions by healthcare providers and policy makers and understanding their strengths and limitations will be critical to their judicious application to treatment and public health strategies.

Legend of Figure

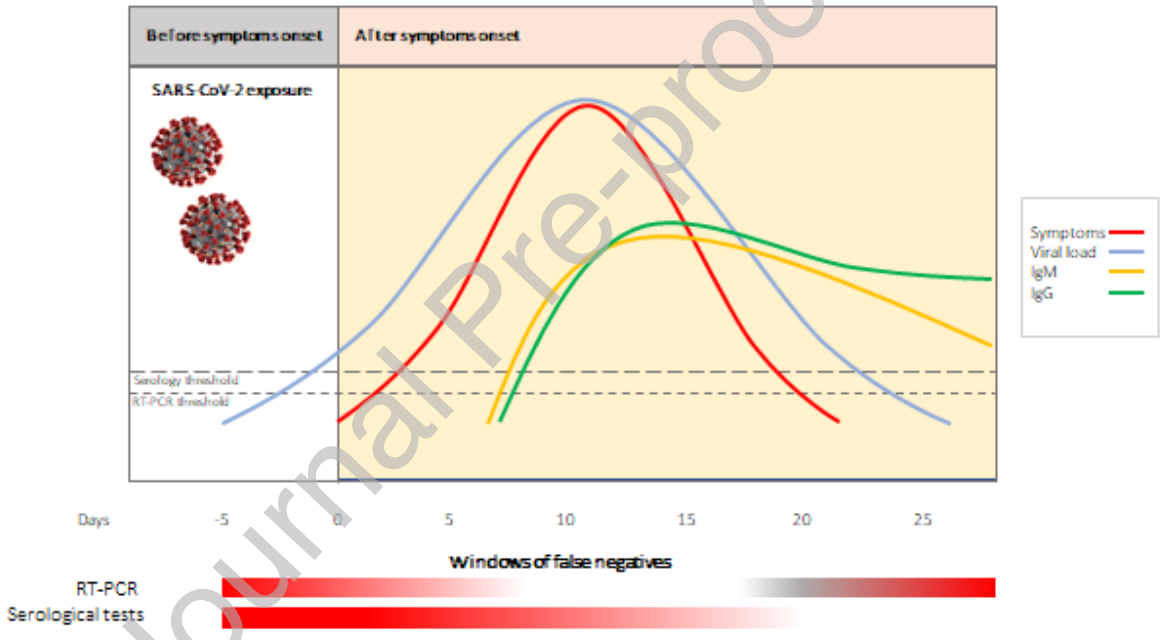

Figure 1 The time-correlation between viral load, symptoms and positivity to the diagnostic tests.

The onset of symptoms (day 0) usually begins 5 days after infection (-5). At this early stage corresponding to the window or asymptomatic period the viral load could be below the RT-PCR threshold and test may give false negative results. As well as at the end of the disease, when the patient is recovering. The seroconversion usually may be detectable 7 to 14 days after the onset of symptoms, hence in the first 12-20 days after the infection the serological tests are more likely to give false negative results.

Table 1 Summary of the original articles reporting on SARS-CoV-2 antibody testing (searched up to May 15, 2020) (Case-reports and review articles have not been included).

PubMed articles											
Author, Year	**Design of the study**	**N**	**Population**	**Nationality of the population**	**Antibody used**	**Methodology**	**Main findings and/or conclusions**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Li Z et al., 2020	Retrospective	525	397 RNA positive Patients, 128 controls	China	Commercial Assay	Jiangsu Medomics Medical Technologies lateral flow immunoassay	The test time was from day 8 to day 33 after infection symptoms appeared. The IgM-IgG combined assay has better utility and sensitivity compared with a single IgM or IgG test. Results demonstrate that the IgG-IgM combined antibody test kit can be used as a point-of-care test	88.66%	90.63 %	NA	NA
Xiao D et al., 2020	Prospective	34	SARS-CoV-2 confrmed patients	China	Commercial assay	Chemiluminescence assay by Shenzhen Yahuilong Biotechnology	After 2 weeks from the onset of symptom, all but two subjects were positive to the test. From the 5^{th} to the 7^{th} weeks IgM became negative, while all had high levels of IgG	94.1%	NA	NA	NA
Zhao J et al., 2020	Prospective	535 samples from 173 subjects	173 RNA positive patients	China	Commercial assay	Beijing Wantai Biological Pharmacy Enterprise ELISA assay	The seroconversion rate for Ab, IgM and IgG was 93.1% , 82.7% and 64.7%, respectively. The cumulative seroconversion curve showed that the rate for Ab and IgM reached 100% around 1 month of illness day.	100% (>15days)	NA	NA	NA
Du Z et al., 2020	Retrospective	60	convalescent patients (6-7 weeks from the	China	Commercial assay	ELISA	All patients tested positive for the IgG against the virus, while 13 patients tested	78% IgM 100% IgG	NA	NA	NA

			onset)				negative for IgM				
Cassaniti I et al., 2020	Prospective	110	30 RNA positive patients, 50 patients with respiratory symptoms, 30 controls	Italy	Commercial assay	Rapid Viva Diag IgM /IgG immunoassay	The rapid test is not recommended for triage of patients with suspected COVID-19 in emergency room	18.4%	91.7%	87.5 %	26.2 %
Guo L et al., 2020	Prospective	208 samples from 140 subjects	82 confirmed and 58 probable cases	China	In House assay	ELISA for IgA, IgM , IgG	IgA, IgM and IgG were detected in 92.7%, 85.4% and 77.9% of samples from a median time of 5 days from the onset of symptoms	75.6% (IgM in confirmed cases) 93.1% (IgM in probable cases)	NA	NA	NA
Jin Y et al., 2020	Retrospective	76	43 RNA positive patients, 33 probable cases	China	Commercial assay	Chemiluminescence Shenzhen YHLO Biotech	Viral serological testing is an effective means of diagnosis for SARS-CoV-2 infection. The positive rate and titer variance of IgG are higher than those of IgM	48.1 % IgM 88.9% IgG	100% IgM 90.9% IgG	NA	NA
Pan Y et al., 2020	Retrospective	105	105 patients	China	In House	Immunocromatography	The positive rates of Ig in the early stage are relatively low, and gradually increase during the disease progression. The IgM positive rate rising from 11.1% of early stage to 74.2% of late stage, respectively. The IgG positive rate in the confirmed patients is 3.6% in early, and 96.8% in late stage, respectively.	68.6%	NA	NA	NA
Padoan A et al., 2020	Retrospective	87 sample from 37 subjects	37 patients	Italy	Commercial assay	MAGLUM 2000 Plus 2019-nCov IgM and IgG assays by Snibe	After the 11th day, all patients were found to be positive for IgG (100%), while the higher positivity of IgM (88%) was	88% IgM 100% IgG	NA	NA	NA

							achieved only after the 13th day. Imprecision and repeatability of the test were acceptable				
Zhong L et al., 2020	Cross-sectional	347	47 RNA positive patients, 300 controls	China	Commercial assay	Elisa and Chemiluminescence detection assay	Both the ELISA and chemiluminescence methods to detect IgG and IgM antibodies by the recombinant N and S proteins of SARS-CoV-2 were consistent	97.9% IgM 95.7% IgG	99.7% IgM 85.7% IgG	NA	NA
Infantino M et al., 2020	Cross-sectional	125	61 RNA positive patients and 64 controls	Italy	Commercial assay	Chemiluminescence (iFlash CLIA)	The ROC auc was 0.918 and 0.980 for anti-SARS CoV-2 antibodies IgM and IgG, respectively	73.3% (IgM) 76.7% (IgG)	92.2% 100%	81.5 % NA	88.1 % 90.1 %
Xiang F et al., 2020	Retrospective	216 samples from 109 subjects	85 confirmed and 24 suspected cases	China	Commercial assays	Zhu Hai LivZon Diagnostics ELISA	The seropositive rate of IgM increased gradually and notably. IgG was increased sharply on the 12th day after onset. Diagnostic performance calculated from samples obtained after 13 days from the onset	77.3 % IgM 83.3% IgG	100% 95%	100 % 94.8 %	80% 83.8 %
Lee YL, et al., 2020	Retrospective	33 samples from 14 subjects , 28 samples from 28 controls	14 RNA positive patients and 28 controls	China	Commercial Assay	Alltest Rapid Test	Antibody response varied with different clinical manifestations and disease severity. Patients with symptoms and development of anti-SARSCoV-2 IgM antibodies had a shorter duration of positive rRT-PCR result and no worsening clinical conditions compared to those without the presence of anti-SARS-CoV-2	78.6%	100%	NA	NA

							IgM antibodies.				
Long QX et al., 2020	Cross sectional	285 patients	285 RNA positive patients	China	Commercial assay	Chemiluminescence Bioscience assay	The positive rate of IgG reached 100% at around 17-19 days after symptoms onset, while IgM seroconversion rate reached its peak of 94.1% at around 20-22 days after symptoms onset	94.% (IgM) 100% (IgG)	NA	NA	NA
Perera R et al., 2020	Retrospective	51 samples from 24 patients	24 RNA positive patients	China	In House assay	ELISA	IgG and IgM were reliably positive after 29 days from illness onset with no detectable cross-reactivity in age-stratified controls.	74%	100%	NA	NA
Qu J et al., 2020	Retrospective	347 samples from 41 patients and 38 samples from controls	41 RNA positive patients and 38 controls	China	Commercial assay	Chemilumenescence, YHLO biotech	The majority of the patients developed robust antibody responses between 17 and 23 days after illness onset	87.8 % (IgM) 97.6% (IgG)	NA	Na	NA
Shen B et al., 2020	Prospective	150 patients	150 suspected cases, of whom 97 were RNA positive	China	Commercial assay	Rapid immunocromatography test by Shanghai Outdo Biotech	The colloidal gold immunochromatography assay for SARS-Cov-2 specific IgM/IgG anti-body shows the potential for a useful rapid diagnosis test for COVID-19.	71%	96%	97%	64%
Zhao R et al., 2020	Retrospective	481	69 affected subjects and 412 controls	China	In House assay	ELISA assay	The overall accuracy of the ELISA test was 97.3%	97.5%	97.5% %	NA	NA
Cai X et al., 2020	Retrospective	276 samples from 276 subjects, 20o samples from 200 controls	276 RNA positive patients, and 200 healthy controls	China	In House assay	Chemiluminescence	Combining immunoassay with real-time RT-PCR might enhance the diagnostic accuracy of COVID-19.	57.2% (IgM) 71.4% (IgG)	NA	NA	NA
Dohla M et al., 2020	Prospective	Samples from 49 symptomatic	22 RNA positive and 27 RNA negative	Germany	Commercial assay	Rapid Test	The rapid test was substantially inferior to the	36.4%	88.9%	72.7 %	63.1 %

		patients	patients				RT-qPCR testing and should therefore neither be used for individual risk assessment nor for decisions on public health measures				
Hoffman T et al., 2020	Cross-sectional	Samples from 153 subjects	29 RNA positive patients and 124 controls	Sweden	Commercial assay	Rapid COVID test by Zhejiang Orient Gene Biotech Co Ltd,	the test is suitable for assessing previous virus exposure, although negative results may be unreliable during the first weeks after infection	69% (IgM) 93% (IgG)	100% (IgM) 99.2% (IgG)	100 % (IgM) 96.4 % (IgG)	93.2 % (IgM) 98.4 % (IgG)
Hou H et al., 2020	Retrospective	338 subjects	338 RNA positive patients	China	Commercial Assay	Elisa test by YHLO	Quantitative detection of IgM and IgG antibodies against SARS-CoV-2 quantitatively has potential significance for evaluating the severity and prognosis of COVID-19.	82.7% (IgM) 88% (IgG)	NA	NA	NA
Imai K et al., 2020	Retrospective	139 samples from 112 patients and 48 controls	112 RNA positive patients and 48 controls	Japan	Commercial assay	One Step IgM/IgG Rapid Test by Artton	Immuno assay had low sensitivity during the early phase of infection, and thus immuno assay alone is not recommended for initial diagnostic testing for COVID-19	40%	NA	NA	NA
Lippi G et al., 2020	Prospective	48 patients	48 RNA positive patients	Italy	Commercial assays	Chemiluminescence MAGLUMI by Snibe and ELISA by Euroimmun	Results of MAGLUMI are well aligned with those of Euroimmun tets	10% (< 5days) 100% (>10 days)	NA	NA	NA
Pan Y et al., 2020b	Retrospective	86 samples from 67 cases	67 RNa positive patients	China	Commercial assay	Rapid Lateral flow assay Zhuhai Livzon Diagnositic	Serology may be considered a supplementary approach in clinical diagnosis	11% (<7 days) 92% (7-14 days) 96%(>14	NA	Na	Na

								days)			
Spicuzza et al., 2020	Cross Sectional	41 subjects	27 RNA positive patients, 7 symptomatic RNA negative patients and 7 controls	Italy	Commercial assay	Rapid lateral flow assay by Beijing Diagreat Biotechnologies	Antibody test is quite reliable and useful, since it has the advantage to be a pointof- care test that gives a response within minutes	83%	93%	NA	NA
Sun B et al., 2020	Cross sectional	130 samples from 38 patients, 16 samples from 16 controls	38 RNA positive patients and 16 controls	China	In House assay	ELISA	IgM and IgG increased gradually after symptom onset and can be used for detection of SARS-CoV-2 infection. Analysis of the dynamics of S-IgG may help to predict prognosis.	75% (after 1 week) 94.7% (after 2 weeks) 100% (after 3 weeks)	NA	NA	NA
To K et al., 2020	Cross sectional	16 patients	16 RNA positive patients	China	In House assay	ELISA	**Serological assay can complement RT-qPCR for diagnosis**	88% (IgM) 94% (IgG)	Na	NA	NA
Xie J et al., 2020	Prospective	56 patients	56 symptomatic patients	China	Commercial assay	Chemiluminescence by YHLO Biological technology	A combination of nucleic acid and IGs testing is a more accurate approach for diagnosing COVID-19	93.7% (IgM) 100 % (IgG)	NA	NA	NA
Yonh G et al., 2020	Retrospective	76 samples from 38 patients	38 symprtomatic patients	China	Comemrcial assay	Rapid assay GICA kit	Antibody detection could be used as an effective indicator os the virus in the absence of viral RNA	50 % (IgM) 92.1% (IgG)	NA	NA	NA
Bryan A et al., 2020	Cross sectional	6001 subjects	1020 controls and 125 patients. 4856 subjects from the general population	USA	Commercial assay	Chemiluminescence by Abbott SARS-CoV-2 IgG test	This study demonstrates excellent analytical performance of the Abbott SARS-CoV2 test as well as the limited circulation of the virus in western	53.1% (day 7) 82.4% (day 10) 96.9% (day 14) 100% (day 17)	99.9%	NA	NA

							United States				
Demey B et al., 2020	Prosepct ive	21 subjects	21 RNA positive patients	France	Comme rcial assays	Four rapid lateral flow assays	The immunochromat ographic tests for the detection of the virus may have their role for the diagnosis of COVID-19	9-24% (day5) 67-82% (day 10) 100% (day 15)	99.8%	NA	NA
Jaaskeil anen A et al., 2020	Retrospe ctive	77 subjects	40 RNA positive patients and 37 controls	Finland	Comme rcial Assay	ELISA by Euroimmun	The median time after onset of symptoms was 12 days (13 patients range: 5–20 days) for detection of IgGs, and 11 days (24 patients range: 5–20 days) for detection of IgAs	na	91.9% (IgG) 73% (IgA)	Na	Na
Montesi nos J et al., 2020	Retrospe ctive	400 subjects	272 controls and 128 RNA positive epatients	Belgiu m	Comme rcial Assays	Chemiluminesce nce by MAGLUMI, ELISA by Euroimmun, and rapid assay	The sensitivity of the tests increased with time from the onset of symptoms	64.3% (MAGLU MI) 84.4% (Euroim mun) 70% (rapid assay)	99% 100%	NA	NA
Tang MS et al., 2020	Retrospe ctive	201 subjects	48 patients and 153 controls	USA	Comme rcial Assays	Chemiluminesce nce by Abbott and ELISA by Euroimmun	Both the two assays have poor sensitivity during the first days of the disease. Abott tests generally performed better than the Euroimmun test	Abbott 0% (<3days) 30% (3-7 days) 47.8% (8-13 days) 93.8% (>14 days) Euroim mun 0% (<3days) 25% (3-7 days) 56.5% (8-13 days) 85.4% (>14 days)	99.4% (Abbot t)	NA	NA

MedRxiv articles											
Author, Year	**Design of the study**	**N**	**Population**	**Nationality of the population**	**Antibody used**	**Methodology**	**Main findings and/or conclusions**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Wang X et al., 2020	Prospective study with longitudinal follow-up	117 samples in 70 subjects	Inpatients and convalescent patients	China	In House	Modified cytopathogenic assay	The seropositivity rate reached up to 100.0% within 20 days since onset. Patients with a worse clinical classification had a higher antibody titer	100%	NA	NA	NA
Garcia PF et al., 2020	Prospective	163	55 RNA positive patients, 63 RNA negative patients, 45 controls	Spain	Commercial Assay	AllTestCOV 19 IgG IgM immunoassay	Sensitivity of the test was 73.9 % after 2 weeks from the onset of the symptoms	73.9%	100	NA	NA
Lassauniere R et al., 2020	Cross sectional	111	30 SARS-CoV-2 patients, 10 healthy controls, 71 patients with non SARS-CoV-2	Denmark	Commercial assays	3 ELISA tests and 6 POC lateral flow tests	The diagnostic performance of the commercial assays analyzed may vary by some degree	65-90% (ELISA) 83-93% (POCs)	96-100 % (ELISA) 80-100% (POCs)	82-100 % (ELISA) 100 % (POCs)	89-98% (ELISA) 80-91% (POCs)
Yangchun F, 2020	Cross sectional	294	186 RNA positive patients, 98 RNA negative patients	China	Commercial assay	ELISA	Antibody testing has a very good diagnostic performance in identifying positive subjects	96.1% (IgG)	92.4% (IgG)	96.09% (IgG)	90.1% (IgG)
Liu R et al., 2020	Retrospective	133	Samples from patients	China	Commercial Assay	YHLO IGs detection kit	In symptomatic patients, the IgM was superior to RT-PCR in detecting affected subjects. The positive ratio for IgM was 79.55% in moderate cases, 82.69% 156 in severe cases and 72.97% in critical cases. IgG antibody test was 93.18% in	78.95% (IgM) 93.18% (IgG	NA	NA	NA

							moderate cases, 100.00% in severe cases and 97.30% in critical cases				
Liu Y et al., 2020	Retrospective	179	Patients RNA positive (n:90) and RNA negative (:89)	China	Commercial assay	Rapid immunoassay	The accuracy of the antibody testing increased over time (from 40% in the first week from the onset of symptoms to 93.9% two weeks later)	85.6%	91%	95.1 %	82.7 %
Yong G et al., 2020	Retrospective	38	Patients	China	Commercial assay	Rapid Assay. GICA IgG IgM detection kit	The accuracy of the test 8 days after the onset of symptoms	50%	92.1%	NA	NA
Lin D et al., 2020	Retrospective	149	79 RNA positive patients	China	Commercial assay	Darui Biotech ELISA kit	The sensitivity of the test increased with time from the onset of the disease	82.2%	97.5%	NA	NA
Lou B et al., 2020	Cross sectional	380	80 RNA positive patients. 300 healthy controls	China	Commercial assay	ELISA and lateral-flow assay	The overall seroconversion rate was 98.8% at a median time of 9 days from the onset of disease	98.8 %	94.3%	NA	NA
Liu L et al., 2020	Cross sectional	238	238 patients, 153 of them RNA positive. 120 controls	China	Commercial assay	Lizhu ELISA assay	Antibody detection should be used as a major viral diagnostic test for patients with symptoms for more than 10 days. The combination of ELISA and RT-PCR assays will greatly improve the detection efficacy, even in theearly stage of infection.	81.5%	NA	NA	NA
Bendavid E et al., 2020	Cross sectional	3300	3300 subjects from the general population	USA	Commercial	Premier Biotech Lateral flow immunoassay	The population prevalence of COVID-19 in Santa Clara- CA ranged from 2.49% to 4.16%, 50 to 85-fold more than reported cases	80.3%	99.5%	NA	NA

Paradiso AV et al., 2020	Prospective	191	191 symptomatic patients	Italy	Commercial	Rapid Viva Diag IgM /IgG immunoassay	The performance of the test at the onset of symptoms was low. The sensitivity was 66.7% 15 days later	30%	89%	NA	NA
Jia X et al., 2020	Retrospective	59	59 suspected patients. 24 of them were RNA positive	China	Commercial assay	Diagreat Immunofluorescence assay	The IgM and IgG may provide a quick, simple and accurate aided detection method for suspected COVID-19 patients	87.5%	NA	NA	NA
Zhang J et al., 2020	Retrospective	736	228 suspected cases, 3 were positive. 508 controls	China	Commercial assay	Chemiluminescence by Shenzhen Yahuilong Biotechnology	Detection of specific antibodies in patients with fever can be a good complement to nucleic acid diagnosis to early diagnosis of suspected cases	100%	97%	75%	100 %
Xiang J et al., 2020	Retrospective	189	154 patients, 35 controls	China	Commercial assays	Zhu Hai Liv Zon Diagnostics ELISA and gold-immunochromatographic assays	There is no difference between the sensitivity of between ELISA and GICA assay they both are simple and fast and the results can be used for clinical reference	87.3% (ELISA) 82.4% (GICA)	100% (ELISA) 100% (GICA)	NA	NA
Hu Q et al., 2020	Prospective	993 samples from 221 subjects	221 hospitalized patients	China	Commercial assay	Chemiluminescence by BioScience	IgG and IgM antibodies examined every 3 days revealed increasing antibody levels which peaked on day 19-21. SARS-CoV-2 IgG and IgM antibodies testing should be combined with RT-PCR as an early diagnosis method	73.6% IgM 97.8% IgG (day 13-18 after the onset)	NA	NA	NA
Ma H et al., 2020	Cross sectional	216 samples from 87 subjects	87 RNA positive patients	China	In House assay	Chemiluminescence	Measuring SARS-CoV-2 specific antibodies IgA, IgM, and IgG in serum provides 81 a better	98.6% IgA 96.8% IgM	98.1% IgA 92.3%IgM 99.8%I	NA	NA

							serological testing with improved sensitivity and specificity	96.8% IgG	gG		
Qian C et al., 2020	Prospect ive, multicen tric	2061 subjects from 10 hospital s	972 non-covid patients, 586 controls, 503 RNA positive patients	China	Comme rcial assay	Chemiluminesce nce by Shenzhen YHLO Biotech	The assay showed a coefficient of variation of less than 5%. SARS-CoV-2 IgM and IgG showed clinical specificity > 97%. 86.54% respectively for suspected cases.	85.8% IgM 96.6% IgG	99% IgM 99%Ig G	NA	NA
Nationa l COVID testing Scientifi c Advisor y Board, 2020	Cross-sectional	182	40 RNA positive patients, 142 controls	UK	Comme rcial assays	Elisa and 9 commercial lateral flow immunoassays (LFIA)	The performance of current LFIA devices is inadequate for most individual patient applications. ELISA can be calibrated to be specific for detecting and quantifying SARSCoV-2 IgM and IgG and is highly sensitive for IgG from 10 days following symptoms onset	85% (ELISA) 55-70% (LFIA versus RT-PCR	100% (ELISA) 65-85% (LFIA versus ELISA)	NA	NA
Burbelo PD et al., 2020	Cross-sectional	100	68 patients, 32 controls	USA	In House assay	Luciferase 44 immunoprecipit ation assay systems (LIPS) to the nucleocapsid (NP) and spike proteins (SP)	Antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than 56 spike protein antibody for detecting early infection.	100% (Ab antin NP) 91% (Ab antiSP)	100% (Ab antiNP) 100% Ab anti SP	NA	NA
Adams ER et al., 2020	Retrospe ctive	841 samples	270 positive samples, 564 negative samples	UK	Comme rcial assay	ELISA by Mologic	The ELISA assay tested had good diagnostic performance	88%	97%	NA	NA
Meyer B et al., 2020	Retrospe ctive	357 subjects	176 controls, 181 RNA positive patients	Switzer land	Comme rcial assay	ELISA by Euroimmun	The assay displays an optima diagnostic accuracy using IgG, with no obvious gain from IgA serology	82%	100%	100 %	46%

Norman M et al., 2020	Retrospective	81 subjects	81 subjects	USA	In House assay	Single Molecular array Assay (SIMOA)	The Simoa serological platform provides a powerful analytical tool	86%	100%	NA	NA
Tuaillon E et al., 2020	Prospective	58	38 RNA positive patients and 20 controls	France	Commercial Assay	Elisa tests by Euroimmun and IdVet and 5 rapid lateral flow tests	The second week of COVID-19 seems to be the best period for assessing the sensitivity of commercial serological assays	86.7 % (ELISA) 80-93.3% (Rapid tests))	80-85% (ELISA) 65-100% (rapid tests)	NA	NA
Wajnberg A et al., 2020	Prospective	1343 subkect	1343 symptomatic subjects, of whom 624 were RNA positive	USA	Commercial assay	Chemiluminescence by Roche	The vast majority of confirmed COVID19 patients seroconvert, potentially providing immunity to reinfection.	82%	Na	NA	NA
Wan Y et al., 2020	Retrospective	180	50 RNA positive patients and 130 controls	China	Commercial Assay	Four Chemiluminescence assay systems	Systems for CoVID-2019 IgM/IgG antibody test may perform differently	26-92%	78-99%	NA	Na
Xiao T et al., 2020	Retrospective	56 subjects	56 RNa positive patients (33 symptomatic and 23 asymptomatic)	China	Commercial assay	Chemiluminescence Microparticle Immuno Assay	Asymptomatic carriers were found to have a lower initial viral load, undetectable IgM and moderate levels of IgG.	90.9% 95.5% 90.9% 63.2%	NA	NA	NA
Zhou Q et al., 2020	Retrospective	419 subjects	19 RNA positive patients and 400 controls	China	Commercial Assay	Chemiluminescence	viral serological testing is an effective means for SARS-CoV-2 infection detection	91.6%	NA	NA	NA
Ozturk T et al., 2020	Cross sectional	148 subjects	32 RNA positive patients, 116 controls	USA	Commercial assay	ELISA by GenScript	The complex relationship between antibody levels, disease severity, and time since symptom onset, caution is needed in using serologic assay to inform public	88.9%	92.3%	NA	NA

							policies				
Rosado J et al., 2020	Retrospective	594	259 RNA positive patients, 335 controls	France	In House assay	Multiplex serological assay Using a serological signature of IgG to four antigens	Serological signatures based on antibody responses to multiple antigens can provide more accurate and robust serological classification of individuals with previous SARS-CoV-2 infection	96.1%	99.1%	NA	NA

NA: not available