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

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Word Count: 9810 (including abstract and references)

Tables:1

Figures: 1

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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.

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.

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