REVIEW ARTICLE

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SEROLOGY AS PRECISE DIAGNOSTIC TOOL IN COVID-19

[1]Siraj Muneer, [2]Shahzad Ali Jiskani

[1]*Department of Chemical Pathology, Aga Khan University Hospital, Karachi*

[2]*Department of Pathology, Indus Medical College, Tando Muhammad Khan*

Corresponding Author:

Siraj Muneer, MBBS, M. Phil (Chemical Pathology), FCPS (Chemical Pathology)
Department of Chemical Pathology
Aga Khan University Hospital, Karachi

Corresponding Author Email:
drsirajmuneer@gmail.com

Co-Author:

Shahzad Ali Jiskani,
MBBS, M. Phil (Haematology)
Senior Lecturer, Department of Pathology
Indus Medical College, Tando Muhammad Khan

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ABSTRACT

Since its origin in Wuhan, China in the last week of December 2019, the coronavirus infectious disease-2019 (COVID-19), caused by the β-Coronavirus, dubbed SARS-CoV-2 has been a global pandemic affecting 212 countries and territories worldwide spanning all five continents. For its management and eradication, prompt and accurate diagnosis of the disease is central. For the diagnosis of SARS-CoV-2 in respiratory clinical specimens, real-time polymerase chain reaction (real-time PCR) using dual labelled TaqMan probe and targeting two genomic areas, typically RdRp and envelope (E) regions, of the virus is commonly used.

Since the stage of the infection cannot be determined during the collection of respiratory nucleic acid test specimens (NAT; RT-PCR), this can lead to false negatives (omission error) as the load of the virus in the respiratory exudates and the saliva progressively decreases with the increase in post-infection time. During clinical sickness that follows an incubation period of normally up to ~14 days, virus excretion will be maximum and clinical samples collected during this period are appropriate for PCR diagnosis than those collected after clinical sickness. In addition, there are other variables that can influence the accuracy of the test result, such as the quality of swabs and virus transport medium, PCR protocol and reagents, enzyme inhibitors, and man power competence engaged in executing diagnostic techniques. In COVID-19, viz., there are three clinical sickness classes: Asymptomatic, symptomatic, moderate and severely symptomatic. Available data indicate that, as was observed in the case of the COVID-19 infected Japan cruise ship 'Diamond Princess' with 3,711 people on board, about 50 percent of people exposed to SARS-CoV-2 infection may become asymptomatic. In the case of asymptomatic and moderate symptomatic cases, due to low virus load in the collected clinical specimens, an effective antibody assay must be used to cross-check the negative result in NAT / PCR. It is understood that with the remission of sickness, the virus

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load in the body and the amount of virus excreted in body fluids steadily decreases, whereas the quantum of particular antibody against the virus increases with time until the plateau. The anti-virus antibody stays in the host for a longer period of time and can be identified even after the infection has been eliminated from the body. NAT must also be accompanied by antibody testing to increase diagnostic efficiency and minimise omission errors. In addition, unlike NAT / PCR, the serology / antibody test is a valuable instrument for controlling the spread of viruses, estimating the actual number of cases and population epidemiological mapping of the disease. In addition, the availability of a precise antibody test system / assay will be useful for COVID-19 post-pandemic surveillance. The current review includes the results of the diagnosis of COVID -19 and antibody response kinetics published by various researchers / groups that support the rapid creation of a 'COVID-19 antibody assay' method for use in disease epidemiological studies.

Key words: COVID-19, Epidemiology, SARS-CoV-2, Polymerase Chain Reaction, Serology.

INTRODUCTION

In humans with a median incubation time of 3 days, SARS-CoV-2 triggers an acute viral infection. [1] Coronaviruses (CoVs) are RNA viruses of single stranded positive sense that occur in four genetic forms, namely, alpha-coronavirus, beta-coronavirus, δ-coronavirus, and gamma-coronavirus. Genetic research has shown that SARS-CoV-2 is a Beta-coronavirus (genus) and a genetic cluster of Sarbecovirus (lineage B), along with certain strains of bat virus with a genetic identity of > 96%. A total of seven CoVs causing mild to severe human disease have been identified; 04 with mild cold-causing seasonal circulation (HKU1, NL63, OC43 and 229E), and the remaining 03 are zoonotic ones, i.e., SARS-CoV (2003), MERS-CoV (2012) and SARS-CoV-2 (2019), originating in various bat species and transmitted to humans through an intermediate host; Civet in the case of SARS-CoV, Dromedary Camel in the case of MERS-CoV, and probably Pangolin [2] in the case of SARS-CoV-2, which has a genetic resemblance of approximately 79% to SARS-CoV and just 50% to MERS-CoV. Structural modelling has shown that SARS-CoV-2 binds to ACE2 with more than 10 times the affinity of SARS-CoV, which explains the faster transmissibility of SARS-CoV-2 in humans compared to SARS-CoV, as well as the higher number of confirmed cases of COVID-19 compared to SARS-CoV. [3] COVID-19 's basic reproduction number (Ro) varies from 2-3.3, which also explains its greater transmissibility compared to SARS and MERS. [4-5] As at 05:31 GMT on 24 May 2020, 28,15,429 COVID-19 cases were involved worldwide, affecting 213 countries and territories spanning all five continents (https:/www.worldometers.info/coronavirus/). As of that date and period, there were 1666828, 349113, 335882, 282370 and 73610 active cases in the USA, Brazil, Russia, Spain and India, respectively. Even after timely diagnosis using nucleic acid tests and introduction of social distancing and lockdowns, this shows active virus transmission. In order to map the population(s) exposed to the virus regardless of the outcome of the infection, it is important to examine the sero-epidemiology of the disease / infection at the earliest using effective antibody tests. In addition, in order to minimise the potential spread of the virus infection by such individuals, NAT negative individuals need to be checked by antibody assay(s). Antibody assays using various viral antigens such as RdRp, nucleoprotein, S1 protein, receptor binding domain (RBD) are used to classify and diagnose infected individuals on a small scale in different countries other than India. Reported

results of antibody assays against RT-PCR in the diagnosis of SARS-CoV-2 infection are compiled in this study.

CELLULAR INFECTIVITY OF CORONAVIRUS

Coronaviruses (CoV) are a wide family of single stranded RNA viruses of positive sense that cause disease in human beings ranging from common cold to more serious diseases such as Extreme Acute Respiratory Syndrome (SARS-CoV of 2003), Middle East Respiratory Syndrome (MERS-CoV of 2012), and Coronavirus Infectious Disease - 2019 (COVID-19). While infections with SARS-CoV and MERS-CoV have a higher mortality rate than COVID-19, SARS-CoV-2 propagates much faster than the two previous diseases. CoVs of various strains have been known to infect and cause illness in poultry, bovine, porcine, canine and feline animals since 1930. SARS-CoV-2 is a novel coronavirus that has not been observed in humans before and has a higher rate of transmission than the two previous CoVs. There are only four structural proteins in the coronavirus: the spike (S), membrane (M), envelope (E) and nucleocapside (N) proteins. The CoV transmembrane glycoprotein (S protein) spikes are highly immunogenic and are an immune response goal. In the S protein, the receptor binding domain (RBD) is particularly targeted by neutralising antibodies. The receptor binding motif (RBM) with both SARS-CoV and SARS-CoV-2 in the RBD region plays a major role in virus neutralisation and only 59 percent is limited to the similarity of the amino acid residues between the RBM of both viruses; neutralising epitopes outside the RBM are also available. [6] On the viral surface, the S glycoprotein is trimeric and mediates the virus's entry into host cells. The S protein has two functional subunits that mediate the attachment of cells (the S1 subunit, consisting of four domains S1A through S1D) and the fusion of the endocytosis-required viral and cell membrane (the S2 subunit). The 1,273-residue SARS-CoV-2 (strain Wuhan-Hu-1) and 1,255-residue SARS-CoV (strain Urbani) spike proteins are 77.5 percent identical and structurally similar in amino acid sequence and bind to the cellular receptor via the S1B domain. Interaction with receptors induces permanent conformational changes in the spike proteins, resulting in endocytosis membrane fusion. [7] Host tropism and virus transmissibility are determined by the S protein. Both SARS-CoV-2 2019 and SARS-CoV 2003 RBD identify and bind to the susceptible cells of the angiotensin converting enzyme 2 (ACE2) receptor, while MERS-CoV binds to the DPP4 (dipeptidyl peptidase 4) receptor. [8-9] SARS-CoV-2 as a whole is genetically distinct from both the 2003 SARS-CoV and the 2012 MERS-CoV. [10]

TESTING FOR COVID-19

COVID-19 testing involves methods for detecting the existence of (i) the genome of the virus by reverse polymerase chain reaction (RT - PCR) or loop - mediated amplification of isothermal nucleic acid (LAMP) and (ii) antibodies produced in response to infection. Antibody detection can be used both for disease diagnosis and for population surveillance. Antibody tests indicate how many individuals are exposed to the infection and can recognise cases that are moderately symptomatic and asymptomatic. The precise estimate of the case fatality / mortality rate (CFR / CMR) of the disease and the population level of herd immunity can only be calculated from the serological survey results of antibody detection. However, since the disease only began in December 2019, the length of the immune response and immunity to COVID-19 is not yet known. For the diagnosis of COVID-19 using respiratory specimens, only

RT-PCR is now being used in the absence of an effective antibody assay system.

POLYMERASE CHAIN REACTION (PCR)

A process that amplifies a given segment of DNA to be detected is polymerase chain reaction (PCR). The SARS-CoV-2 is an RNA virus, reverse transcription polymerase chain reaction (RT-PCR) and its many modifications including Real Time RT-PCR (quantitative PCR) and its further modifications such as Syber green assay calculating amplicon temperature melting (Tm) and TaqMan assay using a dual-labeled probe in addition to 2 primers are used in nasopharmaceutical diagnosis. The probability of detecting the virus in the clinical specimen collected depends on how much time has passed since the individual was infected. In one sample, at week 1 (100 percent), a positive test outcome was highest, followed by 89.3 percent, 66.1 percent, 32.1 percent, 5.4 percent, and 0 percent at weeks 2, 3 , 4, 5, and 6, respectively (Symptom Based Strategy for Discontinuing Isolation for People with COVID-19 (Centers for Disease Control and Prevention, USA, 30 April 2020; SARS-CoV-2 RT-PCR Profile: a preliminary study). Infectious Clinical Diseases. The 19th of April 2020. Doi:10.1093 / cid / ciaa460/5822175).).) Compared to serology for antibody detection for the diagnosis of COVID-19, this genome detection kinetics is the disadvantage of RT-PCR and can lead to omission errors. In a cohort study consisting of 67 patients with COVID-19, the median period of SARS-CoV-2 RNA shedding in nasopharyngeal swabs, sputum, and stools was 12 (3-38), 19 (5-37), and 18 (7-26) days respectively. Just 13 urine (5.6%) and 12 plasma (5.7%) samples were positive for viruses. [11] Another study showed that viral RNA detection based on RT-PCR is sensitive and can confirm early SARS-CoV-2 infection effectively. [12-13] A cohort study [14] of 23 laboratory-confirmed COVID-19 patients (median age 62 years [range 37-75]) conducted at two hospitals in Hong Kong during January-February 2020 revealed a median viral load of 5•2 log10 copies per ml in the posterior oropharyngeal saliva or other respiratory specimens. The saliva virus load was the strongest within the first week after symptom onset and subsequently decreased with time. Viral RNA was observed 25 days after the start of symptoms in one patient. A higher viral load was associated with older age. The outcome of the PCR test is determined by the quantity of viral load in the specimen.

SEROCONVERSION AND ITS DETECTION

Reverse transcriptase polymerase chain reaction (RT-PCR) has regularly been used for its diagnosis since the beginning of COVID-19. Several authors have, however, pointed out the poor performance of this technique, particularly in terms of sensitivity; RT-PCR sensitivity may be as low as 38%. [10, 15-16] Serology was used as a supplementary assay to RT-PCR for the identification of anti-viral IgM / IgG. [17-18] RT-PCR detects only the genome of the virus, while antibody tests are helpful in testing the spread of the population as it shows exposure to the virus, and the antibody isotype (IgG / IgM) detected speaks about the time of infection with the virus. According to the WHO, seroconversion is the transition from seronegative status (no antibodies in the serum or present but below the detection limit) to seropositive status in which serum samples can detect antibodies. Isotype-switching, also called switching of the immunoglobulin class, is the transfer from one type to another of antibody development by B cells. The first antibodies to be produced against an antigen are IgM isotype antibodies, then the isotype changes to IgG antibodies, which are more

effective for immune defence. The isotype(s) of the antibody present in a serum / specimen patient may provide useful information on the timing of initial exposure to the virus, as well as information on disease progression and prognosis. IgM suggests new infection, and previous infection or convalescence is indicated by IgG. Detection of virus – specific antibodies is essential for [1] diagnosis of suspected cases with negative RT-PCR results, [2] identification of asymptomatic infection, and [3] monitoring of virus transmission and sero-surveillance in the target population to understand virus circulation. [12-13]

Serology was mainly an epidemiological method in the case of the SARS-CoV epidemic (2003-04), and could help assess the number of silent infections, disease development, patterns of virus spread, and the probable origin of the virus. [19] In order to better estimate the number of COVID-19 cases, including those that may be asymptomatic or have recovered (FDA, USA), antibody testing for SARS-CoV-2 is in increased demand. Serology tests will assess whether, by looking at their immune response, individuals have been exposed to a specific pathogen. RT-PCR tests currently used globally for the diagnosis of COVID-19, on the other hand, can only indicate the existence of the viral genome during infection and do not indicate whether a person has been infected and has subsequently recovered. By recognising individuals that have produced antibodies to the virus, antibody testing may provide greater information on the prevalence of a disease in a population. [20] Antibodies cannot be identified early in the infection and this restricts the efficacy of serological assays for COVID-19 diagnosis. [20] Serological examination, however, may play a critical role in recognising individuals who have previously conquered an infection and developed an immune response. No nation has accurate data on the prevalence of the virus in its population due to insufficient serological testing. Serological testing can be helpful for the diagnosis and detection of asymptomatic infections of suspected patients with negative RT-PCR results. Confirming reported cases of COVID-19 as early as possible using serological testing could reduce the risk of repeated sampling exposure and save valuable RT-PCR tests. [12-13] Seven cases with no symptoms and a negative RT-PCR result were positive for IgG and/or IgM antibodies in this report, which illustrates the significance of serological testing in achieving more reliable COVID19 pandemic scale estimates.

A human monoclonal antibody (MAb 47D11) that neutralises SARS-CoV-2 and SARS-CoV has been reported for the first time. [21] MAb 47D11 binds to an RBD (spike protein receptor binding domain) conserved epitope and neutralises both SARS-CoV and SARS-CoV-2 via a mechanism that is independent of the inhibition of ACE2 receptor binding. For the development of antigen detection tests and serological assays targeting SARS-CoV-2, this MAb will be useful.

ISOTYPES AND THEIR DIAGNOSTIC SIGNIFICANCE

- In early infection, IgM antibodies are developed
- In later infection, IgG antibodies are developed, and are also commonest antibody isotype in blood and other fluids of body. The IgG antibodies provide defence against infection and the immune system also has memory.

Local / mucosal immunity is associated with IgA antibodies and is located on the mucous membranes of the lungs, sinuses, stomach and

intestines. They are also present in blood, as well as in saliva and tears.

SEROLOGY ASSAY TYPES

- **Rapid Diagnostic Test: This is a qualitative lateral flow assay (positive or negative) used for the identification of antibodies (IgG and IgM) or viral antigens. IgM / IgG antibodies against nucleoprotein (N / NP) of SARS-CoV-2 are detected by available test systems.**
- **Enzyme - Linked Immunosorbent Assay (ELISA): This test can be qualitative or quantitative and can make use of patient samples of whole blood, plasma, or serum. It is possible to detect antibodies (IgM / IgG) against spikes (S) (either S1 or S2 as a whole or RBD), N and M.**
- **Neutralization Assay: This test detects serum / plasma antibodies that are successful against the virus in clearing up the infection. Several modifications to this test are accessible.**
- **Chemiluminescent-Immunoassay:This test is quantitative, and in whole blood, plasma, and serum, various forms of immunoglobulins like IgG, IgM, and IgA can be identified.**

VIRAL ANTIGEN DETECTION

ELISA can detect a particular viral antigen. The problem with the antigen detection system is that there may often be insufficient antigen present in the nasal swab to be detectable, particularly in asymptomatic individuals. There is no amplification procedure for viral proteins in an antigen test, unlike the RT-PCR test. The sensitivity of antigen detection tests for respiratory diseases such as flu ranges from 34 percent to 80 percent, according to the WHO, and half or more of COVID-19 infected patients could be missed by such tests, leading to omission errors. However, 91.7 percent (11/12) of patients were able to detect the virus in saliva in certain trials. [14]

ANTIBODY DETECTION KINETICS

SARS-CoV-2 IgG antibodies are normally detectable 10-14 days after infection, and typically peak about 28 days after infection. It is possible to detect IgM antibodies earlier. Since antibodies take time to develop, they are not the best indicators of acute infection, but they are ideal for detecting past infections / convalescence as they can remain in the bloodstream for several years. Anti-N / NP IgM could be observed on day 7 and day 28 in a cohort study consisting of 67 COVID-19 patients, while IgG was on day 10 and peaked on day 49 after disease onset. In extreme patients, IgM and IgG titers were significantly higher than in non-severe patients ($p < 0.05$). The length and essence of immunity against infection with SARS-CoV-2 is not yet understood. [11] The median antibody detection time for SARS-CoV-1 (12 days; IQR 8-15.2 days) and SARS-CoV-2 (11 days; IQR 7.25-14 days) was similar, but for MERS-CoV (16 days; IQR 13-19 days) was longer. [22] There was no detectable cross-neutralization against SARSCoV-2 by SARS patient serum. [23]

A analysis of acute SARS-CoV-2 antibody responses in 285 COVID-19 patients showed that 100% of patients tested positive for antiviral immunoglobulin G (IgG) within 19 days of symptom onset. [12-13] The severe group had higher IgG and IgM titers than those in the non-severe group. Serological testing can be helpful for the diagnosis and detection of asymptomatic infections of suspected patients with negative RT-PCR results. Seroconversion occurred concurrently or sequentially for IgG and IgM, and both titres of IgG and IgM were plateaued within 6 days of seroconversion (loc.

cit.).

The seropositivity score was 94 percent for anti-NP IgG, 88 percent for anti-NP IgM, 100 percent for anti-RBD IgG, and 94 percent for anti-RBD IgM in serum samples available from 16 patients for 14 days or longer after symptom onset. [14, 24] Increases in IgG or IgM antibody levels against NP / RBD were observed in most patients 10 days or later after symptom onset. More patients experienced earlier anti-RBD seropositivity than anti-NP.

In female patients, the IgG antibody production was higher than in male patients at the early stage of the disease. [25] Although the underlying mechanisms are not understood, this difference in the level of IgG antibodies between male and female patients can contribute to negative clinical outcomes in male patients with COVID-19.

SEROLOGICAL ASSAYS

Serology was mainly used as an epidemiological method in the case of the SARS-CoV outbreak of 2003 that could help identify inapparent infections, disease progression mechanism, viral transmission pattern, and the probable origin of the virus. [19] Analysis found that patients with COVID-19 had IgM seroreactivity at day 4 after onset of symptoms, which peaked at day 9, while IgG increased dramatically 12 days after onset of symptoms, and all patients with viral nucleic acid were positive for IgG 30 days after onset of symptoms. [18] IgM antibodies were found in 87.5 percent and IgG in 70.8 percent of cases in patients suspected of COVID-19 and tested negative for the viral genome. They showed that COVID-19 diagnostic sensitivity was 77.3 percent for IgM with 100 percent precision, compared to 88.3 percent and 95 percent for IgG, respectively. In the case of COVID-19 diagnosis using the technique of virus genome detection, the test result can be affected by pre-analytical variables such as inconsistency in obtaining nasopharyngeal swabs, the different swabs and transport medium used, time and temperature of transport of specimens, and potential presence of nucleic acid / PCR inhibitors in the sample, etc. [26] Serological data analysis can be useful for evaluating exposure to the virus, but serology may be more difficult for patients with acute infection to be interpreted; cross-reactivity with other coronaviruses and pathogens may be an issue. [19, 26] The speed of diagnosis of COVID-19 infected patients can be improved by coupling the possible shortcomings and strengths of both viral genome detection and serological assays. [18] This research (loc. cit.) is a first step towards a deeper understanding of the antibody response to SARS-CoV-2 and offers valuable insight into the potential characteristics and usage of COVID-19 pandemic serological tests. A SARS-CoV-2 S1 serology ELISA kit was developed using the full length SARS-CoV-2 S1 protein expressed by CHO cell as the capturing antigen. The precision of this ELISA (means negative as negative) and sensitivity (means positive as positive) were 97.5% and 97.1%, respectively, with an overall accuracy rate of 97.3%. [27] On the first day after the initiation of the disease, the assay was able to detect SARS-CoV-2 anti-bodies and was able to detect particular antibodies in 28 out of 276 asymptomatic individuals and in one out of five PCR-negative near contacts of COVID-19 patients.

The presence of IgM would mean a recent infection, while a prior infection would indicate IgM negative and IgG positive. This monitoring technique will be most successful 1-2 weeks after the initial onset of symptoms, as well as helping to determine the immunity of herds and the possibility of new infections for those who

are returning from quarantine. The sensitivity of the antibody test ranged from 28.7 percent (symptom onset 1-7 days) to 73.3 percent (symptom onset 8-14 days) and 94.3 percent at symptom onset for more than 15 days. [28] During the first 7 days of symptom initiation (ranging between 67-72 percent), molecular tests have restricted sensitivity, which could be due to low viral load early in the course of the disease or variations in the selection technique. [28]

For large-scale sero-epidemiology studies, the use of RBD-IgG ELISA as a screening test for SARS-CoV-2 antibody, followed by confirmation using the plaque reduction neutralisation test, was adapted to evaluate population infection attack rates and identify disease severity and herd immunity. [29-30] A positive RBD ELISA outcome was predictive of a previous SARS-CoV-2 infection. Large-scale sero-epidemiologic studies will provide near real-time population infection attack rates. [31]

The potential role of IgM antibodies against SARS-CoV-2 as a diagnostic marker of recent in-fection has been assessed by recent studies. [32] Using an ELISA using SARS-CoV-2 recombinant NP antigen, it was shown that IgM antibodies were detectable in 85% of COVID-19 confirmed patients 1-7 days after symptom onset. [33] These authors suggested that while molecular testing remains preferred, with higher sensitivity, IgM targeting may be useful in suspected COVID-19 patients diagnosed as negative by molecular methods within the first 5.5 days after disease onset. Just about 28 percent of patients could detect IgM antibodies against RBD found in the S1 subunit of the virus spike glycoprotein by day 7 of post-symptom onset, while 73 percent were positive by day 14. [34] Recent studies have shown that IgA antibodies against the virus are detectable as early as one day after the onset of symptoms, close to IgM. [33] During the COVID-19 pandemic, identification of IgG antibodies against the virus may have a greater role to play in comparison to IgM and IgA isotypes. In addition, the long-lasting IgG response is close to that of IgA and is correlated with viral neutralising activity, which is important for disease recovery. [35-36] Serologic monitoring for the identification of IgG isotype antibodies against the virus will play an important role in determining the true prevalence of the virus. [32] Studies have also indicated a relatively high specificity of IgG-based serological assays for COVID-19 (> 95 percent). [12-13, 32, 37] Data indicated that IgG formed against various SARS-CoV-2 antigens was detectable in patients after at least 8 days after clinical disease, and more than 90% of patients were seropositive after day 14 of disease, while some individuals may take longer to become seropositive, depending on their immune status, or may never be seropositive if immunosuppressed significantly. [12-13, 34]

Neutralizing anti-bodies were detectable in 89% of patients up to 2 years after infection from previous immunity studies in recovered SARS-CoV patients, whereas IgG antibodies were undetectable at 6 years of age. [38-39] With respect to SARS-CoV-2, we have to wait until that time to have similar results. In different populations and exposure scenarios, the rate of asymptomatic COVID-19 infection has been estimated at 4 to 80 percent, and seroprevalence studies will therefore help to create a more reliable estimate of the number of infected individuals that will in turn help to determine the true case fatality rate (CFR) at regional, national and global levels. [40-42] Serological tests assess the proportion of people exposed to the virus. Early studies have indicated that detection of IgM and IgG usually occurs between 7-11 days after exposure in COVID-19 patients. The outcome of immunochromatography

and chemiluminescent immunoassay for the detection of SARS-CoV-2 antibodies was not affected by heat inactivation of blood samples at 56°C for 30 min, but may raise the risk of infection for laboratory staff handling the tests. [43]

ASYMPTOMATIC CASES

A cruise ship, the 'Diamond Princess' housing 3,711 individuals, was quarantined for 2 weeks on 5 February 2020 after COVID-19 was diagnosed with a passenger going ashore. 634 people on board tested positive for SARS-CoV-2 before 20 February 2020, of which 306 were symptomatic and the remaining 328 were asymptomatic (50.5%). [3] There are two forms of asymptomatic cases of SARS-CoV-2 infection, i.e., 1) individuals with minor or moderate symptoms during the incubation period but with initiation of symptoms during the quarantine period, and 2) individuals with no symptoms all the time but positively screened for viral nucleic acid or antibodies. Asymptomatic infection is a problem, and super spreaders are considered asymptomatic individuals. Those with mild to no symptoms but positive for SARS-CoV-2 viral nucleic acid or positive for serum specific IgM antibodies are asymptomatic carriers. [3] There is evidence suggesting potential transmission from asymptomatic cases of SARS-CoV-2. The viral load detected in asymptomatic patients was close to that detected in symptomatic patients, indicating the potential for asymptomatic or minimally symptomatic patients to undergo transmission. [44] In faeces, but not in nasopharyngeal swabs, an asymptomatic case tested positive for the virus indicates a theoretical possibility of transmission through a faeco-oral path. [45] Suspected asymptomatic patients should be quarantined and tracked for 14 days, and their quarantine will end if two consecutive nucleic acid test samples obtained at intervals of more than 24 hours are negative. [46] In order to assess the number of individuals infected with little or no symptoms and estimate the actual number of reported cases, the application of antibody tests is appropriate for screening various age groups of people. With the presence of differences in the actual number of asymptomatic cases and their infectivity, it is important to elucidate broader observational and longitudinal studies utilising serological tests. [31] Strict quarantine of asymptomatic patients, however, is of great importance in managing the COVID-19 pandemic worldwide, and if several feasible transmission control steps are taken, then the epidemic may end rapidly and effectively. [3]

ASSAY FOR HOST IMMUNE RESPONSE

Myxovirus resistance protein A (MxA) has a low baseline (less than 15 ng / ml), long half-life (2.3 days) and rapid induction (1-2 hours) biomarker for viral infection. [47] MxA mRNA has been shown to be detectable in peripheral blood within 1-2 hours of interferon (IFN) alpha-stimulated white blood cells, and then MxA protein starts to accumulate. [48] In the case of MERS-CoV and SARS-CoV, these coronaviruses have been shown to increase the expression of MxA in the blood. [49] Many studies have shown that peripheral blood MxA protein expression is a responsive and precise marker of viral infection. MxA protein expression is regulated solely by type I IFNs. [47, 50] The MxA gene is expressed in mononuclear blood cells or locally in tissues, and other cytokines such as IL-1 or TNF-alpha apparently do not react to the MxA gene. [51]

RECOMBINANT VIRAL PROTEINS

Now that the SARS-CoV-2 genome sequence is known, it is possible to generate the viral

protein(s) of interest as a recombinant protein in E. Coli or Eukaryotic / Baculovirus systems for use in ELISA in large quantities. After the antigen is directly bound to the wells, a human serum test is added and a secondary antibody (usually labelled with the HRP enzyme) is added that responds to human antibodies (in the test serum) bound to the antigen, and a colorimetric or fluorometric output can be quantified by the presence of the mark (HRP etc.) in the secondary antibody.

CONCLUSION

In order to complement COVID-19 diagnosis by RT-PCR, the use of precise antibody assay systems is a must, as there are chances of false positives (in PCR tests) due to variability in virus load in the clinical materials collected for diagnosis. The stage of infection during the processing of clinical samples for nucleic acid tests is difficult to guarantee. During clinical illness, the virus load in respiratory exudates is likely to be maximal, and steadily decreases with sickness remission. Therefore, in nucleic acid studies, samples obtained late in the infection will turn out to be negative. However, antibodies elicited following infection with the virus can be detected for longer periods of time, and asymptomatic cases can also also be diagnosed by antibody testing. Serology, unlike NAT, is beneficial in understanding the spread of the virus and the disease's epidemiology.

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