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REVIEW ARTICLE

Rapid Serological Testing for Managing the COVID-19 Pandemic: A Review

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Abstract:

With the onset of the novel coronavirus disease pandemic (COVID-19) that emerged from Wuhan in China, the need of the hour can be summarized into two groups. The first one is a potent vaccine as a prophylactic measure to prevent the virus from infecting people, and the second is a rapid diagnosis of the disease to help healthcare professionals and government authorities to plan and control the spread and provide effective care and treatment. This review delves into the latter, describing the COVID-19 and its treatment, including the race for an effective vaccine, and highlighting the role of serological testing in managing the pandemic since a well-designed study to understand mechanisms and serological correlations of protective immunity is crucial for rational clinical and public health policies. In conclusion, swift vaccination and response tactics, such as social distancing, hand hygiene, wearing of masks, and, if required, lockdown practices continue to be important in managing the pandemic while carefully monitoring any possible outbreak due to the variants.

Keywords: SARS-CoV-2, Pandemic, COVID-19, Serological testing, Seroprevalence, Vaccine.

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1. INTRODUCTION

Coronaviruses (CoV) are a family of RNA viruses that typically cause mild respiratory disease in humans [1]. According to the Center for Disease Control and Prevention (CDC) Jan 2020 report, there are seven coronaviruses known to infect humans, of which only MERS-CoV and SARS-CoV are routinely capable of causing severe respiratory infections. The recent one is a novel SARS-CoV-2 identified in Wuhan, China, in December 2019 [2]. The emergence of human coronaviruses, which is thought to have been directly transmitted from bats to humans, is also theoretically possible. The existence and identity of an intermediate host for SARS-CoV-2 remain to be explored and are still unclear [3]. On 30th January 2020, World Health Organization (WHO)declared the recent coronavirus outbreak as a public health emergency of international concern and subsequently declared it as a pandemic on 11th of March 2020. As of June 2021, more than 184 million cases and 3,994,988 deaths have been reported worldwide due to the SARS-CoV-2 pandemic (worldometers.info/coronavirus). Consequently, stringent infection control is critical to prevent transmission.

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Chu *et al.* in their systematic review and meta-analysis, supported physical distancing of more than 1-meter, compulsory use of face masks, and eye protection as a means of transmission control in healthcare and community settings. Hence, strict social distancing through implementing a lockdown is a very important control strategy.

As predicted by German researcher Lothar Wieler, president of the Robert Koch Institute- Germany, many regions are bracing up for second and even a third wave of infections [4]. Hence, it is very urgent and important to continue exploring means and methods of monitoring spread and preventing the infection through various interventions that include the discovery of diagnostics, the development of drugs, and to be future-ready through the development of prophylactic vaccines.

This review provides an overview of COVID-19, its causative agent, the SARS-CoV-2, drugs, and prophylactic vaccines to combat the disease and discusses seroprevalence to highlight the significance of such assays in monitoring the spread and management of the pandemic. Seroprevalence is the number of persons in a population who test positive for the SARS-CoV-2 based on serological antibody tests, often expressed as a percentage of the total population.

2. ORIGIN OF THE COVID-19

The outbreak of disease probably started from single or multiple zoonotic transmission events in Wuhan [5]. This group of viruses contains a very large RNA genome with a complicated life cycle. Ordinarily, this group causes seasonal colds in humans and is mostly not a serious illness. A variant caused Severe Acute Respiratory Syndrome (SARS) around 2003 and killed a few thousand people, and then disappeared. Later MERS emerged from camels in the Middle East and killed a few hundred people around 2012. Now, we are witnessing the emergence and spread of the COVID-19.

Beginning innocuously in December 2019 as a cluster of pneumonia cases in Wuhan of Hubei province in China (Archived: WHO Timeline-COVID-19, April 2020), SARS-CoV-2 has emerged as a major pandemic with a death toll in the immunologically naïve human population has reached a staggering 784,380 worldwide by 19th of Aug 2020. The death rates from the SARS-CoV-2 have varied from around 2% in China to approximately 10% in Italy and Spain. While the early clinical symptoms are typical of respiratory infections and pneumonia, death usually occurs from underlying co-morbidities, and complications arise after both clinical symptoms and viral titers have waned, suggesting a critical role for immunopathology. As reported in previous SARS-CoV and MERS-CoV human infections, the SARS-CoV-2 also causes mild to moderate illness in younger people below 50 years of age while leading to severe manifestations in older people and those with comorbidities such as diabetes, obesity, heart failure, and renal failure. This pandemic is the third instance of a coronavirus threatening the health of our world. The SARS-CoV in 2002-03 and the MERS-CoV of 2012 mercifully spared us from a full-blown global pandemic [6, 7].

2.1. Structure and Classification of SARS-CoV-2 Virus

The novel coronavirus 2019 (COVID-19), also known as the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), is a single and positive-stranded RNA virus that belongs to the order Nidovirales and family Coronaviridae with its genome size varying from 29 to 32 kilobases [8, 9]. The SARS-CoV-2 shares 79.5% and 96% genome similarity with SARS-CoV and bat Coronavirus, respectively [10, 11]. The order of genes/coding regions of SARS-CoV-2 is as follows: replicase ORF1ab, spike (S), envelope (E), membrane (M), and the nucleocapsid (N). It has also been confirmed that SARS-CoV-2 uses the same cell entry receptor, angiotensin-converting enzyme II (ACE2), as SARS-CoV [10]. The earliest publications from Chinese laboratories which sequenced the viral RNA from the early clinical cases in Wuhan revealed that the 30,474 nucleotides long SARS-CoV-2 showed a nucleotide identity of 89.1% with a bat SARS-like coronavirus (CoV) isolate, bat SL-CoVZC45 (GenBank accession number MG772933), that had previously been sampled in China [11, 12].

2.2. Diagnostic Efforts

Current RT-PCR (real-time reverse transcriptase-polymerase chain reaction) amplification of the viral DNA is considered the most reliable testing method. However, it is not always positive in the COVID-19 patients [13]. The computerized tomographic (CT) imaging of the chest could play an important role in detecting lesions in the parenchyma of lungs in the suspected patients. Hence, examination of a chest CT along with the RT-PCR results is recommended for precise diagnosis [14].

Based on the data gathered by Artis Ventures, as of 19th August 2020, a total of 375 diagnostic tests have been authorized by regulatory bodies such as FDA (Food and Drug Administration) and EEA (Europe Economic Area). Out of the 375 tests, about 193 use PCR (Polymerase Chain Reaction), and around 138 are serological assays. Around 163 molecular assays have a downtime of 2 to 8 hours to show results, and 37 assays show results in 1 to less than 1 hour. Some of the assays include but are not limited to RealTime-SARS-CoV-2 assays from Abbot (giving results in 4-6 hours), Cobas-SARS-CoV-2 test from Roche (giving results in 3-8 hours), and Linea COVID-19 Assay kit from Applied DNA Sciences showing results in 1 hour [15]. The goal of all the diagnostic tests is to be accurate, quick, and cost-effective, which will help the patients get the care and the treatment they need in a timely manner and quickly rule out others who do not carry the disease [16]. In return, this could also help prevent the spread of disease by allowing the infected patients to be quarantined or in self-isolation.

2.3. Therapeutic Efforts

So far, no drugs or biotherapeutics are approved by the FDA to prevent or treat COVID-19. Currently, Remdesivir [17], a broad-spectrum antiviral drug first used to treat the Ebola virus outbreak, has obtained emergency use authorization (EUA) from the FDA in May 2020, based on preliminary data showing a faster recovery time of patients with severe conditions [18, 19]. In June 2020, Russia approved the generic drug Favipiraviras the most promising anti-COVID-19 drug for its anti-viral properties [20]. Other drugs for treating symptoms, anti-inflammatory agents, and those for prevention are under various stages of clinical trials [15]. Prominent drugs and their mode of action are summarized in Table **1**.

2.4. Prophylactic Vaccines

With the rapid spread of SARS-CoV-2, the search for an effective and permanent cure is still going on, with vaccines being developed by almost every country. Since SARS-CoV-2 shows similar pathways with SARS and MERS viruses, researchers use the knowledge gained from these two pathways as a basis to develop a vaccine against SARS-CoV-2. Researchers worldwide are working towards the development of vaccines. At least 20 teams aim to develop nucleic acid (DNA or RNA) vaccine coding for a coronavirus protein (mostly virus's spike protein) to prompt an immune response. At the same time, many other researchers are engaged in making a sub-unit vaccine (a coronavirus protein directly injected into the body) [21]. Artis ventures in its website www.av.co/covid mentioned that 12 DNA, 10 inactivated, 3 live attenuated, 19 non-replicating viral vectors, 18 replicating viral vectors, 54 protein subunits, 17 RNAs, and 14 virus-like particle vaccines are under various stages of development.

According to WHO, several vaccine candidates are in various stages of clinical trials, while 19 are approved by at least one country for emergency use purposes (covid19.trackvaccines.org/vaccines/). A version of the live and attenuated BCG (Bacillus Calmette – Guerin) vaccine is also considered the potential therapeutic approach to protect healthcare workers and vulnerable individuals by reducing the impact of the SARS-CoV-2 [22].

Table 1. List of prominent drugs considered in the COVID-19 treatment.

Drug	Mode of Action
Remdesivir	Inhibition of viral replication by the termination of RNA replication
Favipiravir	Inhibition of viral replication by inhibiting RNA Polymerase activity
Lopinavir/Ritonavir	Protease inhibitor blocks viral replication
Umifenovir (Arbidol)	Intercalates into membrane lipids and blocks fusion of membranes with viral particles
Chloroquine	Inhibition of viral entry into host cells by interfering with the ACE2 cellular receptor and by impairing endosome acidification
Oseltamivir	Interferes with the release of viral particles from cells by inhibition of viral neuraminidase
Ribavirin	A ribonucleotide analog that inhibits viral replication by interfering with viral RNA synthesis and viral mRNA capping

There are many research teams along with companies and universities worldwide in the race for an effective vaccine. Several groups have already begun phase III clinical trials; others have started testing in animals. At least seven teams have developed vaccines using the virus itself in a weakened or inactivated form. Around 25 groups claimed that they are working on viral-vector vaccines (a weakened virus is genetically engineered so that it can produce coronavirus proteins in the body). At least 20 teams aimed to develop nucleic acid (DNA or RNA) vaccine coding for a coronavirus protein (mostly virus's spike protein) to prompt an immune response. At the same time, many other researchers are engaged in the process of making a sub-unit vaccine (a coronavirus protein directly injected into the body) [21].

According to WHO, at least 7 vaccine candidates have completed the phase III stage of the development (Table **2**). A vaccine called ChAdOx1 nCoV-19, developed by Oxford university-UK and AstraZeneca, is reported to be highly promising by generating antibodies and T-cells against the SARS-CoV-2 virus successfully in 1077 volunteers [23]. It is made from a genetically engineered virus that causes the common cold symptoms in chimpanzees. Furthermore, Chinese firm Sinopharm in collaboration with United Arab Emirate's artificial intelligence and cloud computing company called Group 42 and Abu Dhabi Department of Health, have announced phase IV trials for an inactivated vaccine (covid19.trackvaccines.org/vaccines/). The third one, CoronaVac (PiCoVacc), is developed by Sinovac, which has also begun phase IV trials for the inactivated form of the virus. This vaccine is inactivated by formalin and combined with an alum adjuvant. It is similar to the vaccine Sinovac invented against the SARS virus in 2002 [24]. Since India will be one of the biggest consumers of the COVID-19 vaccine, India's Bharat Biotech Company, in alliance with the Indian Council of Medical Research (ICMR) – National Institute of Virology (NIV), is developing a COVAXIN vaccine candidate. Covaxin has demonstrated an encouraging positive safety profile in the phase-I trials (clinicaltrialsarena.com/news/bharat-biotech-covaxin-data/). Phase-II clinical trials of Covaxin were conducted at Gauhati Medical College & Hospital (GMCH), Assam. Recently, Russia has approved the world's first COVID-19 vaccine, dubbed Sputnik V, tested in 76 people. The vaccine was developed by the Gamaleya Research Institute of Epidemiology and Microbiology in Moscow and is now in the phase III trials.mRNA-1273 is another one that has begun phase III trials, which is developed by Cambridge, Massachusetts-based company called Moderna, Inc., and the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH). The vaccine is a synthetic mRNA and is designed to produce antibodies against SARS-CoV-2 [25]. Finally, in recent days, a vaccine, BNT162b2, which is developed by Pfizer (United States of America) and BioNTech (Germany) in collaboration with Forsun Pharma (China), has begun phase III trials in Germany. Out of the 4 mRNA vaccines used for phase I and II trials, Pfizer-BioNTech-Forsun selected BNT162b2 because of its tolerability profile andT-cell responses against the receptor-binding domain (RBD) and the spike glycoprotein of the SARS-CoV-2 virus (SE, 2020). In March 2021, the FDA issued an emergency use authorization to Johnson and Johnson, USA, of a one-shot vaccine called Janssen Covid-19 vaccine, which is also an adenovirus-based vaccine.

Artis ventures in its website www.av.co/covid mentioned 12 DNA, 9 inactivated, 3 live attenuated, 19 non-replicating viral vectors, 17 replicating viral vectors, 50 protein subunits, 16 RNA, and 12 virus-like particle vaccines that are under various stages of development.

Table 2. List of 39 vaccines in Phase III of clinical trials (covid-19.trackvaccines.org).

S. No.	Vaccine	Type	Company	Approval Status Till June 2021
1	Soberana Plus (Finlay-FR-1A)	Protein Subunit	Instituto Finlay de Vacunas, Cuba	Not yet approved
2	Recombinant (Sf9 Cell)	Protein Subunit	West Chain Hospital, China	Not yet approved
3	Covovax	Protein Subunit	Serum Institute, India	Not yet approved
4	Abdala (CIGB-66)	Protein Subunit	Center for Genetic Engineering and Biotechnology, Cuba	Not yet approved
5	Sanofi/GSK Recombinant Protein	Protein Subunit	Sanofi Pasteur	Not yet approved
6	SCB-2019	Protein Subunit	Clover Biopharmaceuticals, Australia	Not yet approved

(Table 2) contd.....

S. No.	Vaccine	Type	Company	Approval Status Till June 2021
7	UB-612	Protein Subunit	Covaxx, USA	Not yet approved
8	NVX-CoV2373 (Covovax)	Protein Subunit	Novavax, USA	Not yet approved
9	EpiVacCorona	Protein Subunit	FBRI at Kobe	Approved in Russia and Turkmenistan
10	Nanocovax	Protein Subunit	Nanogen	Not yet approved
11	RBD-Dimer (ZF2001)	Protein Subunit	Anhui Zhifei Longcom	Approved in China and Uzbekistan
12	Soberana 02 (FINLA-FR-2)	Protein Subunit	Instituto Finlay de Vacunas, Cuba	Not yet approved
13	Plant-Based VLP	Virus-like particle	Medicago	Not yet approved
14	AG0302-COVID19	DNA	AnGes	Not yet approved
15	ZyCoV-D	DNA	Zydus Cadila	Not yet approved
16	INO-4800	DNA	Inovio Pharmaceuticals	Not yet approved
17	BNT162b1	RNA	Pfizer/BioNTech	Not yet approved
18	Walvax Covid-19 Vaccine	RNA	Walvax Biotechnology	Not yet approved
19	mRNA-1273 (Spikevax)	RNA	Moderna	WHO emergency use listing; Approved in 58 countries
20	mRNA-1273.211	RNA	Moderna	Not yet approved
21	CVnCoV	RNA	Curevac	Not yet approved
22	BNT162b2	RNA	Pfizer/BioNTech	WHO emergency use listing; Approved in 94 countries
23	Ad5-nCoV (Convidecia)	Non-Replication Viral vector	CanSino	Approved in 8 countries
24	Covisheild	Non-Replication Viral vector	Serum Institute, India	WHO emergency use listing; Approved in 44 countries
25	Ad26.COV2.S (Ad26COVS1, JNJ-78436735)	Non-Replication Viral vector	Johnson & Johnson	WHO emergency linting; Approved in 55 countries
26	GRAd-COV2	Non-Replication Viral vector	ReiThera	Not yet approved
27	AZD1222 (Vaxzevria)	Non-Replication Viral vector	Oxdford/Astra Zeneca	WHO emergency use listing; Approved in 118 countries
28	Sputnik light	Non-Replication Viral vector	Gamaleya	Approved in 10 countries
29	Sputnik V	Non-Replication Viral vector	Gamaleya	Approved in 69 countries
30	Inactivated (Vero cells)	Inactivated virus	Chinese Academy of Medical Sciences	Not yet approved
31	ERUCOV-VAC	Inactivated virus	Health Institutes of Turkey	Not yet approved
32	COVIran Barekat	Inactivated virus	Shifa Pharmaed Industrial Co.	Approved in Iran
33	BBIBP-CorV	Inactivated virus	Sinopharm (Beijing)	WHO emergency listing; Approved in 56 countries
34	Inactivated (Vero Cells)	Inactivated virus	Sinopharm (Wuhan)	Approved in China
35	CoronaVac	Inactivated virus	Sinovac	WHO emergency listing; Approved in 37 countries
36	VLA2001	Inactivated virus	Valneva	Not yet approved
37	QazVac (QazCovid-in)	Inactivated virus	Kazakhstan RIBSP	Approved in Kazakhstan
38	Covaxin	Inactivated virus	Bharat Biotech, India	Approved in 9 countries
39	Kconvac	Inactivated virus	Minhai Biotechnology Co	Approved in China

2.5. Convalescent Plasma Therapy

Convalescent Plasma therapy (CP) is a type of immunotherapy that has been used to effectively treat infectious diseases for over a century. Patients were treated with satisfactory efficacy and safety for diseases, like SARS, MERS, and H1N1 pandemic [26 - 29]. With the non-availability of an approved drug or vaccine to treat the COVID-19 disease, convalescent plasma therapy is being considered along with the standard treatment for the severe cases of COVID-19. Patients with a high neutralizing antibody titer against the SARS-CoV-2 virus are a valuable source of CP [30]. However, based on the published literature, high-titer convalescent plasma therapy has no significant impact on 28-day mortality or clinical recovery [31].

Convalescent plasma has the potential to show some benefit for patients with impaired humoral immunity. In a recent case series of 23 patients with prolonged COVID-19 on anti-CD20 agents who received a transfusion of a high-titer unit, 87% became PCR-negative and experienced full clinical recovery [32, 33]. In August 2020, the Food and Drug Administration (FDA) announced an Emergency Use Authorization for convalescent plasma in the COVID-19 patients. Later in February 2021, FDA limited its use only to hospitalized COVID-19 patients early in the course of disease and patients with impaired humoral immunity.

2.6. Herd Immunity

When more people are exposed to the virus, some manifest

it clinically, whereas others may be sub-clinically, and thus a community starts building immunity at large; in either case, it is called herd immunity. Herd immunity, if developed, can have a multiplier effect. It can protect many people. However, herd immunity can work only when there is some vaccination also. However, recently, there is a report of pre-existing immune reactivity in the general population [34], which could be due to exposure to common cold coronaviruses. This finding may have implications for herd immunity and vaccine development. Most estimates had placed the herd-immunity threshold at 60–70% of the population, either through vaccinations or past exposure to the virus [35].

2.7. Serological Testing

Detection for specific antibodies in blood samples is referred to as serological testing. Antibodies, also called immunoglobulins, are proteins produced in our body in response to an antigenic substance particularly from pathogenic microorganisms such as bacteria and viruses. They circulate in our system as a defense towards subsequent infection with the same pathogen. They are rapidly produced by the B cells of the immune system and can be detected in our blood approximately in a week's time after symptoms begin to occur. There is an increased demand for serological testing for SARS-CoV-2 since it provides better ways to quantify the number of cases and has emerged as a promising solution to identify individuals exposed to the novel coronavirus and suffered from novel coronavirus disease. The presence of anti-COVID-19 antibodies can help medical practitioners make a conclusive diagnosis of the COVID-19, especially, when the nasopharyngeal swab tests are negative. Moreover, antibody testing can be useful in understanding those with mild symptoms of the flu and the asymptomatic carriers who may not show any sort of flu symptoms. Testing for the COVID-19 is typically done using polymerase chain reaction (PCR) with a nose or throat swab. However, the amount of detectable virus decreases after about five days and could be more difficult to test through this technique. Furthermore, the RT-PCR tests currently being used globally to diagnose the COVID-19 can only indicate the presence of viral material during infection and will not indicate if a person was infected and subsequently recovered. Also, this test is often positive in individuals without any symptoms.

Serological assays are easy to perform, less expensive, and have a short waiting period as compared to RT-PCR, nucleic acid assays. These assays, like enzyme-linked immunoassay (ELISA) for specific IgM and IgG antibodies, have a greater advantage such that they avoid false-negative results unlike RT-PCR [36]. In a recent report, patients who tested COVID-19 positive via the RT-PCR method were divided into two groups based on the time between the onset of disease and testing. It was found that seven out of ten patients in the first group and 13 out of 19 patients in the second group tested IgM positive within 9-17 days and 18-29 days, respectively. Nine out of ten patients in the first group and 18 out of 19 patients in the second group were tested IgG positive [37]. This indicates that the major limitation of serological assays is that the antibodies appear later during the disease. According to Zhong *et al.* [38], ELISA and chemiluminescence methods to detect IgG and IgM antibodies by the recombinant N and S proteins of SARS-CoV-2 were more consistent with the nucleic acid detection assay. In addition, a survey conducted by Long *et al.* [39] found that patients who tested PCR negative and had no COVID-19 symptoms showed a positive in IgG/IgM assay, indicating how important serological assays are to determine the extent of the COVID-19 pandemic, especially in asymptomatic cases.

Serology-based tests can give greater detail into the prevalence of a disease in a population by identifying individuals who have developed antibodies to the virus. Serological testing is comparatively rapid and is critical in understanding the spread of the virus via asymptomatic individuals and provides an understanding of the expected time of protection. It has the potential to contribute to the efforts in the current pandemic response [40]. However, the understanding of long-term protection *via* immune response to the COVID-19 is still in the early stages since not all antibodies produced are the same [41]. Also, reports suggest that the required performance characteristics of antibody tests will critically depend on individual-level vs. population-level studies [40]. Understanding the epidemiology of the disease, degree of prevalence, and the development of immunity is possible only from seroprevalence studies including testing for IgM and IgG against the virus due to long-lasting antibodies [42].

Furthermore, a minimum load of antibodies in our body is required to determine the level of protection. The level of protection directly correlates with the number of antibodies and this would necessitate procedures on how to integrate those who have recovered from the virus back into society. Also, long-term surveillance is still a problem since it is not yet established whether patients with antibodies can contract a second SARS-CoV-2 infection. In their review article, Zainol Rashid *et al.* [43], based on the performances of nine rapid detection tests (RDTs) including the evaluation reports concluded that antibody-based serological assays improve diagnosis of positive cases. In Italy, the patient was declared clear of the virus when the RT-PCR accompanied by the specific IgG antibody test showed negative results.

In one review from Stanford University, the author examined six systematic evaluations of seroprevalence data and concluded that the SARS-CoV-2 infection is widely spread and suggested only a 0.15% average global infection fatality rate (IFR) with substantial heterogeneity.

2.8. Performing the Tests

The *in-vitro* testing involves combining viral components such as viral spike proteins and others with the patient's blood sample. If the blood contains antibodies to the virus, they will recognize and bind specifically to the viral component. Then the plate is washed, and the bound antibodies are determined. Most of the antibody tests detect IgG antibodies to the Spike (S) protein of the virus, although other isotypes and antigens are being investigated. The testing methods are based on ELISA, flow cytometry and chemiluminescence, and immuno-chromatography principles of which immunochromatographic assay is very well recognized for self-testing. The mere

presence of antibodies may not signify immunity and does not necessarily mean that they are neutralizing the virus.

2.9. Basic Methods of Available Serological Assays

2.9.1. Lateral Flow Rapid Serology Test

This is a rapid qualitative assay that is cost-effective, portable, and can be used at the point of care (POC). The test yields a positive or negative result using a peripheral blood sample, saliva sample, or a small quantity of nasal swab fluid. The test is performed in a plastic cassette and shows up colored lines indicating a positive or negative result. These tests are developed mostly for the subject's IgG and IgM antibodies or viral antigens.

2.9.2. Enzyme-linked Immunosorbent Assay (ELISA)

This test can be a qualitative or quantitative *in-vitro* assay that can be performed on whole blood, serum, or plasma samples. The test plate is coated with a viral protein, such as Spike protein, and is incubated along with the patient sample. The presence of any antibodies (IgG and IgM) to the viral protein will result in specific binding. The bound antibody-viral protein complex is then detected with the help of other antibodies that produce a color or fluorescence which can be read out.

2.9.3. Neutralization Assay

This type of assay is primarily performed to know if a patient has active and effective antibodies against the virus even after recovery from infection. This test relies on antibodies that prevent infection of cells *in-vitro*. The test is performed on a whole blood sample, serum sample, or plasma sample obtained from a patient. Neutralization assays depend on cell cultures to allow the growth of the SARS-CoV-2 virus. Viruses and cells are grown along with decreasing concentrations of antibodies to quantify antibodies in the serum that block viral replication by binding to an important viral protein that enables entry into the cell.

2.9.4. Chemiluminescent Immunoassay

This is an *in-vitro* quantitative assay performed on a sample of whole blood, plasma, or serum obtained from the patients. The test involves mixing patient samples with the known concentration of viral protein, buffer reagents, and specific enzyme-labeled antibodies that allow detectable luminescence. Occurrence of antibodies in the sample results in antibody-viral protein complex formation which is detected by the addition of secondary enzyme-linked antibodies inducing a chemiluminescent reaction. The amount of chemiluminescence is then used to quantify antibodies present in the sample. This test can help identify IgG, IgM, and IgA types of antibodies.

2.10. Integration of Antibody Testing for Effective Surveillance and Containment

Several questions remain unclear at this stage. The reports may tend to be negative when there could be a deeper lower respiratory infection. Also, it is not yet clear a) whether people can get re-infected, or will the immune response give them long-term protection, and b) whether people infected will have resistance to other strains of the virus. Hence, though there could be short-term protection, there is no guaranteed long-term immunity at this point in time. With respect to a safe return to work, the protocol is never going to be 100% perfect. Thus, it depends on the answers that emerge in due course of time for the above queries. Antibody testing may help reduce false-negative results and provide reliable data for correlation purposes. According to a report, a foolproof return-to-work protocol is dependent on determining a minimal level of antibody protection [41]. With emerging COVID-19 data, we probably can determine the total risk and the amount of risk society is willing to accept. Multiple waves of the COVID-19 are anticipated [44]. In the meantime, health care workers are gathering as much data as possible to better inform standard operating procedures of sick patients, determine therapeutic interventions, and develop a vaccine.

Data from China and elsewhere suggested the fact that most people have shown mild to moderate symptoms when they contract COVID-19. Serum-based antibody studies are very important to quantify infection in the community and understand the number of mild and asymptomatic cases. This knowledge helps authorities plan for future interventions against the COVID-19. A response to a later reappearance could be tailored to protect only high-risk people if we know that a high percentage of people in a community were infected in its first wave of infection [45]. Reliable serologic evidence of immunity at the individual level will have important implications for the conditions under which individuals return to work, and their ability to work in settings at high risk of transmission, particularly certain health care settings.

However, there are still uncertainties about the characteristics of immunity, including durability and completeness of protection. These questions will need to be settled before serostatus could be considered for individual decision-making. Evidence on the sensitivity and reliability of new serologic tests must be evaluated before they are integrated into health care policies.

2.11. Path Ahead

Decisions on how to contain the COVID-19 pandemic without completely shutting down business operations require reliable data and associated tools to understand the extent of transmission. Health care systems are heading to develop and implement serological testing for clinical use, evaluate social policy, and quantify risk at the level of the population. This has highlighted the challenges of surveillance programs globally. Hence, it is necessary to revisit and reevaluate surveillance data generated by our health systems and the way in which we can conveniently apply serological testing.

Rapid testing for active cases and tracing of contacts by directly testing for the presence of the virus is a key for containment strategies. There is a pressing need for real-time monitoring of changes in disease transmission across populations and geographies. For later phases of pandemic control, serological testing is important in understanding transmission intensity and population susceptibility which

guide decisions on when, where, and how to lift lockdowns and relax social distancing constraints. As highlighted by Bryant *et al*., challenges to the assessment of performance characteristics of these serological rapid tests are threshold concerns on the sensitivity and specificity, the potential cross-reactivity with other coronaviruses, the use of neutralization assays as a gold standard, difficulties in harmonizing results reporting across different platforms, concerns for quality control in manufacturing, and lack of baseline data required for test interpretation. Moreover, the performance of different test formats may considerably vary, for instance, point of care lateral flow assay is likely to be marked with sensitivity and specificity concerns than ELISA format. However, the low cost and ease of use will facilitate ELISA format for rapid scale-up and widespread adoption. Despite efforts to study immune responses to the COVID-19, to date, there is a lack of sufficient and reliable data on the magnitude and duration of IgM, IgG, and IgA responses in severe, mild, and asymptomatic cases. Furthermore, it is not yet clear how antibody responses vary among diverse populations with different genetic backgrounds and comorbidities.

Use for individual *versus* population-level IgG testing applications emphasized the dangers of serologic testing for individual-level risk assessments but have highlighted the potential of population-level serological testing, referred to as seroepidemiology, even with assays of moderate sensitivity and specificity. For use at the individual level, many of the serological tests have concerns over the sensitivity and specificity characteristics. Hence, WHO has currently restricted antibody testing to research purposes only. Nevertheless, these tests provide valuable inputs on public health concerns such as the safety of relaxing lockdowns or evaluations of alternative intervention measures. Many fundamental questions relating to testing performance, the dynamics of antibody responses in relation to infection, and the link between antibody responses and immunity need to be addressed to realize the benefits of population-level seroepidemiological studies. Answers to these questions across different populations and epidemiologic contexts call for study designs that are key to the optimal interpretation of the growing number of population-based, cross-sectional serological surveys [40].

While many countries are gradually gearing up to learn to live with the virus, several tests, strategies, and research are being conducted to ensure a cure for the COVID-19. Serological tests can also serve to decode the immunity status in regional populations and answer questions on whether a country or region has attained the COVID-19 peak and whether a strategy, as a lockdown, is effective. For example, as indicated in Table **3**, one such serological survey undertaken in the city of Mumbai, India, on the population of 6936 individuals revealed that 40.5% of the subjects have developed antibodies against SARS-CoV-2 [46, 47]. A similar survey conducted by the Delhi government again in India on random samples from 22823 individuals revealed that at least 23.48% of them were already infected. A high seroprevalence means that a larger population may be immune to SARS-CoV-2 and may break the chain of infection.

Table 3. Percentage seroprevalence of SARS-CoV-2 antibodies in selected countries.

Place	Date	% Seropositive	Data Source
USA	Mar-May 2020	Ranged from 1-6.9% from 10 sites (Avg. 3.21%)	Havers *et al.*, 2020 [48]
Germany	April 2020	15%	Vogel, 2020 [49]
Mumbai, India		40.5%	Hingorani *et al.*, 2020 [47]
Delhi, India	June-July 2020	23.48%	pib.gov.in, 2020 [50]
Spain	May 2020	5%	Yasinski, 2020 [51]
Geneva, Switzerland	April-May 2020	8.3% (Avg. of 5 weeks)	Stringhini *et al.*, 2020 [52]

These surveys may inform us of the transmission pattern from region to region, especially from slums to plush localities and from country to country [52 - 54]. And if new vaccines practically prevent deaths, then the global IFR may decrease even below 0.1% [55 - 56].

CONCLUSION

While antibody testing is crucial in the assessment of the SARS-CoV-2 exposure, infection, and potential immunity, detection of asymptomatic infection is critical for understanding the overall prevalence and infection potential of the COVID-19 and determining the rate of increased seroprevalence with the progress of the pandemic. Serological rapid tests are subject to reader training for reliable performance and are suitable for screening purposes. The extent and time course of antibody development is yet to be understood and may vary between different populations and even among RT-PCR results. At present, there is no gold standard test to identify true seropositive. Surveillance by serological tests has the potential to provide reliable cumulative infection rates but requires thorough evaluation covering the entire spectrum of the SARS-CoV-2 infections, from symptomatic and mild to severe infection and later convalescence. Since the sensitivity of the serological test is a function of test time from the onset of disease, such tests provide an overall picture of seropositivity rather than dynamic time-dependent trends in individual antibody titers. This may be necessary to evaluate the value of serology in arriving at an accurate estimate of the cumulative infection rate. A well-designed study to understand mechanisms and serological correlations of protective immunity is crucial for rational clinical and public health policies. In addition, rigorous validation of the current standard assays of the COVID-19 positive cases is necessary. Furthermore, the assays are needed to be foolproof and validated with samples from individuals with other viral infections since cross-reactivity may result in false positives and compared them with the samples of individuals before the COVID-19 outbreak to understand specificity. Sero-surveys are thus important in every country or region to better understand the overall prevalence.

Finally, since the pandemic is ongoing, it is imperative that

response tactics such as hand hygiene, compulsory wearing of masks, isolation of infected individuals, quarantine, social distancing, and community containment practices are to be implemented while authorities should carefully monitor for any possible outbreaks due to mutations causing an antigenic conversion.

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CONFLICT OF INTEREST

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