REVIEW

Serological assays and host antibody detection in coronavirus-related disease diagnosis

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Abstract

Coronaviruses (CoV) are a family of viral pathogens that infect both birds and mammals, including humans. Seven human coronaviruses (HCoV) have been recognized so far. HCoV-229E, -OC43, -NL63, and -HKU1 account for one-third of common colds with mild symptoms. The other three members are severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV-2. These viruses are responsible for SARS, MERS, and CoV disease 2019 (COVID-19), respectively. A variety of diagnostic techniques, including chest X-rays, computer tomography (CT) scans, analysis of viral nucleic acids, proteins, or whole virions, and host antibody detection using serological assays have been developed for the detection of these viruses. In this review, we discuss conventional serological tests, such as enzyme-linked immunosorbent assay (ELISA), western blot (WB), immunofluorescence assay (IFA), lateral flow immunoassay (LFIA), and chemiluminescence immunoassay (CLIA), as well as biosensor-based assays that have been developed for diagnosing HCoV-associated diseases since 2003, with an in-depth focus on COVID-19.

Introduction

Virus-induced diseases are pervasive worldwide, ranging from mild infections to lethal diseases. Among the diverse families of viruses is the family *Coronaviridae*, of the order *Nidovirales*. *Orthocoronavirinae* is a subfamily of the family *Coronaviridae* whose members infect birds and mammals, including humans [1, 2]. Human coronaviruses (HCoVs) are responsible for various respiratory diseases, e.g., common colds, bronchitis, and pneumonia [3]. Urban sprawl and farming have contributed to the rapid pace at which these viruses have been evolving [4]. In general, coronaviruses (CoVs) are enveloped viruses with a non-segmented, positive-sense single-stranded RNA genome that is approximately 30 kb in length and encodes four structural proteins; the membrane (M) glycoprotein, the small envelope (E) protein, the spike (S) glycoprotein, and the nucleocapsid (N) phosphoprotein, which encapsulates the viral RNA. The M and E proteins are vital for viral envelope formation and maintenance of virion structure [5, 6]. The S protein, which has a molecular weight of about 180 kDa and a length of 20 nm, consists of two subunits, S1 and S2. The S1 subunit, which contains the receptor-binding domain (RBD), recognizes and binds to its receptor angiotensin-converting enzyme 2 (ACE2), which is found in human lungs. The S2 subunit, by undergoing specific conformational changes, induces membrane fusion, enabling viral entry into the host cell. This protein is the chief target of neutralizing antibodies, which prevent infection and further dissemination by blocking binding to ACE2. The 40- to 60-kDa N protein is the viral protein that is produced and shed in the largest quantities during infection [7–11].

In the case of SARS-CoV-2, the immune system initially generates an uncontrolled response through the hyperactivation of monocytes and macrophages, which leads to an increase in the number of neutrophils and interleukin 6 (IL-6) and a reduction in the lymphocyte population. For the adaptive immune response, T and B lymphocytes are necessary for cell-mediated and humoral immunity, respectively. In cell-mediated immunity, cytotoxic T cells identify and eradicate infected cells, while in humoral immunity, B cells produce neutralizing, immunoglobulin G (IgG), and immunoglobulin M (IgM) antibodies that block viral particles from binding to host cells

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[12, 13]. IgG and IgM antibodies are generally produced shortly after exposure to the virus, with IgG usually being secreted later than IgM. Zhou et al. demonstrated that the maximum IgM level is reached 9 days after infection and that secretion of IgG antibodies begins during the second week [14, 15]. These antibodies are commonly produced against the viral S, E, or N proteins [16–18].

In severe COVID-19 cases, a condition termed "cytokine storm" has been observed. This syndrome results from an excessive uncontrolled inflammatory response that leads to the overproduction of pro-inflammatory cytokines, particularly IL-6. Patients experiencing a cytokine storm may suffer from acute respiratory distress syndrome (ARDS), tissue damage, or organ failure, which can have a fatal outcome [19].

To date, seven CoVs have been identified in humans, including HCoV-229E and -OC43 in the 1960s, SARS-CoV in November 2002, HCoV-NL63 in 2004, HCoV-HKU1 in 2005, MERS-CoV in June 2012, and SARS-CoV-2 in December 2019 [5, 14, 20]. HCoV-229E, -OC43, -NL63, and -HKU1 account for one-third of common colds, often producing mild symptoms, but in some cases, they can cause bronchitis or pneumonia [21]. SARS-CoV, MERS-CoV, and SARS-CoV-2, which are more likely to produce severe symptoms, are responsible for SARS, MERS, and COVID-19, which are life-threatening infectious respiratory tract diseases [22].

More than 8000 SARS-associated cases and 770 deaths were reported during the 2002-2004 SARS epidemic in 29 countries, with a case-fatality rate of roughly 9%. The last SARS-related case was reported in April 2004, and since then, no new cases have been reported [23–25].

MERS-CoV, which is closely related to *Tylonycteris* bat CoV HKU4 and *Pipistrellus* bat CoV HKU5, may infect humans via direct or indirect contact with dromedary camels. Human- and camel-derived MERS-CoV have shown more than 99.5% nucleic acid sequence identity. Since its outbreak in June 2012 until the end of January 2020, 2519 confirmed MERS-related cases and 866 deaths have been reported. The case-fatality rate was approximately 34%, which is significantly higher than that of SARS [26, 27].

It is widely believed that SARS-CoV-2 was initially transmitted to humans via contact with animals at the Wuhan live animal and seafood market in China in December 2019. Since the genome sequence of this virus is approximately 89% identical to that of the bat SARS-like CoV ZXC21 and 96.2% similar to that of the bat CoV RaTG13, researchers have proposed that SARS-CoV-2 is of bat origin. Moreover, its genomic sequence is 79.6% identical to that of SARS-CoV, resulting in it being renamed from "2019 novel CoV" (2019-nCoV) to "SARS-CoV-2". By the end of August 2020, the ongoing COVID-19 pandemic resulted in more than 25 million confirmed cases and 850,000 deaths in over 213 countries and territories worldwide [15, 28–34].

In addition to the chest X-rays and CT scans employed to diagnose CoV-related diseases, several other techniques are used to identify the virus directly, by analyzing the viral RNA or proteins or the whole virus. These direct analytical methods include nucleic acid amplification tests (NAATs), such as reverse transcription polymerase chain reaction (RT-PCR) and isothermal nucleic acid amplification assays, ultrasensitive amplification-free RNA biosensors, and whole-virus or viral proteins assays [35–42, 121]. Furthermore, host antibody detection, which is the main focus of this review, is another technique that indirectly identifies the virus, by detecting antibodies that recognize viral particles [43, 44].

The detection of host antibodies produced against viral antigens in serum samples is a well-established method for determining the presence or absence of CoVs. These tests, known as serological assays, are frequently used by clinicians due to their rapidity and low cost when compared to the RT-PCR technique recommended for viral RNA detection. However, the sensitivity of these assays highly depends on the time of viral exposure, which determines the antibody level. These tests are also incapable of determining the precise time of disease onset [45, 46]. Serological assays may be used to determine the portion of the population who have had the disease and recovered from it and are therefore potentially immune to the infection. Although it has been suggested that screening for these antibodies might be used to identify individuals for whom it is safe to return to work [47], on April 24, 2020, the World Health Organization (WHO) stipulated that even people who have recovered from COVID-19 and tested positive for antibodies are not necessarily protected from becoming reinfected. One reason for this uncertainty was the low levels of neutralizing antibodies observed in the sera of several recovered COVID-19 patients, which implied that in addition to humoral immunity, cell-mediated immunity might have been crucial to their recovery [48]. Therefore, more consideration should be given to the potential applications of serological testing.

Most HCoV-specific antibody detection assays target IgG and IgM antibodies, but there have been tests targeting immunoglobulin A (IgA) antibodies as well [18, 49–53]. The capture antigens in these assays are either whole virus particles from cultured infected cells, the viral membrane S or E proteins, or the N proteins [43].

Apart from the two label-free surface plasmon resonance (SPR)-based optical biosensors, most serological tests have utilized the conventional label-based enzyme-linked immunosorbent assay (ELISA), western blot (WB), immunofluorescence assay (IFA), lateral flow immunoassay (LFIA), and chemiluminescence immunoassay (CLIA) techniques [16, 17, 54–57]. In this article, we classify the host antibody detection assays into two types: (1) conventional serological tests and (2) biosensors. We then review the studies and

commercial assays of each classification. A summary of the reported studies to date for the detection of host antibodies against CoVs is presented in Table 1.

Conventional serological assays

Traditional serological tests regarding CoV-related disease diagnosis mostly rely on the well-known labeled ELISA, WB, IFA, LFIA, and CLIA methods [53, 55, 57–59], which are extensively discussed in the following sections. As shown in Table 1, after the SARS outbreak, between the years 2003 and 2013, more than 20 studies were conducted that incorporated these assays to detect host antibodies against SARS-CoV in human serum or plasma samples. Additionally, in 2005 and 2006, seven assays were reported for identifying antibodies against HCoV-OC43 and -229E. After the emergence of MERS in 2012, three serological tests were developed to identify antibodies against MERS-CoV, and since the ongoing COVID-19 pandemic, 11 studies have been reported regarding anti-SARS-CoV-2 detection.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA technique is a quantitative analytical method that identifies the presence or absence of a specific analyte in a given sample by measuring its concentration via antigen-antibody interactions, which lead to a color change in the presence of an enzyme label and its related substrate [60]. The analyte may be either an antigen or, in the case of serological ELISAs, antibodies [43]. ELISA-based serological tests for CoV-associated diseases have employed horseradish peroxidase (HRP) or alkaline phosphatase (AP) as the enzyme labels, using appropriate substrates [61, 62]. The peroxidase substrates exploited in these assays include 3, 3, 5, 5'-tetramethylbenzidine (TMB), *o*-phenylenediamine dihydrochloride (OPD), and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) [53, 63, 64].

ELISA is a heterogeneous enzyme-based immunoassay that can be classified into four distinct types: (1) direct, (2) indirect, (3) sandwich, and (4) competitive [60]. Serological assays reported for CoV detection have used indirect, sandwich, and competitive ELISA principles [44, 64, 65]. As illustrated in Fig. 1A, in an indirect ELISA, a diluted or undiluted serum sample containing the target antibodies is added to antigen-coated plates, where targets form complexes with the capture antigens. After washing to remove unbound molecules, HRP- or AP-labeled detection antibodies are added that recognize the primary or target antibodies. Following further incubation and washing steps, a substrate is added that is converted by the conjugated enzyme to produce a visible color. The intensity of the color of the reaction

product is directly correlated with the concentration of the analyte [66].

In a sandwich ELISA-based serological test, target antibodies are recognized by the capture antigens or antibodies in the first step. Subsequently, the enzyme-labeled detection antigens identify the antigen-antibody or capture-target antibody complexes and bind to their target antibody segments. Following the addition of the enzyme-specific substrate, the concentration of the target corresponds directly to the color intensity [60, 66].

As depicted in Fig. 1B, in a competitive ELISA, a serum sample containing the target antibodies and HRP-labeled analogs of the targets are added simultaneously to antigen-coated plates. The targets and analogs compete for the immobilized capture antigens. After the introduction of the peroxidase substrate, the color intensity is measured, which is inversely proportional to the concentration of the target antibodies [67].

As shown in Table 1, 14, one, and 13 distinct ELISA assays have been reported for detection of antibodies against SARS-CoV, MERS-CoV, and SARS-CoV-2, respectively. The MERS diagnostic assay is a competitive ELISA [64]. The IgM-specific test developed by Xiang et al. for SARS-CoV-2 detection and two of the assays proposed by Zhao et al. for COVID-19 diagnosis involving the simultaneous identification of IgG and IgM antibodies and the individual detection of IgM antibodies are sandwich ELISAs [58, 65]. The other assays are based on the indirect ELISA principle. Except for the serological test developed by Zhao et al. for IgM antibody detection, all of the assays detect IgG antibodies [65]. Nine of these assays were designed to identify both IgG and IgM antibodies, and five detected IgG, IgM, and IgA antibodies. The time required for these assays varied from 1 hour to 4 hours and 10 minutes [50, 53].

In 2004, Guan et al. developed two ELISA platforms for the detection of anti-SARS-CoV antibodies. Their assays detected IgG antibodies against the viral N protein within 1 hour and 15 minutes, with a sensitivity of 70.5-100%. Furthermore, they evaluated the tests using a commercial IFA as the gold standard [68, 69]. Liu and colleagues also designed a 1-hour-and-20-minute ELISA procedure to detect IgG antibodies against the SARS-CoV N protein. The assay was able to detect the target antibodies in 1:1000-diluted sera [70].

In 2004, Woo and coworkers reported an ELISA incorporating N proteins as the capture antigens for the detection of SARS-associated IgG, IgM, and IgA antibodies at serum dilutions of 1:40, 1:10, and 1:10, respectively. The assay had a 3-hour-and-15-minute duration. Also, in 2005, they presented two ELISA-based tests. Both assays detected IgG and IgM antibodies in serum samples, one employing the S protein, and the other, the N protein as the immobilized capture molecule. Under optimal conditions, the S-protein-based test took 3 hours and 15 minutes and detected the antibodies

Table 1 Reported assays for HCoV-specific antibody detection

Virus	Analyte	Detection method	Assay time	Limit of detection	Tested sample	Reference
Conventional serological assays						
SARS-CoV	IgG antibody	IFA	-	1:1600 dilution	Serum	[97]
SARS-CoV	IgM, IgG, and IgA antibodies	ELISA	-	1:1000 dilution	Serum	[49]
		IFA		-		
SARS-CoV	IgG antibody	IFA	-	-	Serum	[98]
SARS-CoV	IgG antibody	ELISA	1 h 20 min	-	Serum	[99]
SARS-CoV	IgG antibody	IFA	1 h 30 min	1:40 dilution	Serum	[86]
SARS-CoV	IgG antibody	ELISA	1 h 15 min	-	Serum	[68]
		LFIA	15 min			
SARS-CoV	IgG antibody	ELISA	1 h 15 min	-	Serum	[69]
		LFIA	15 min			
SARS-CoV	IgG antibody	WB	2 h 30 min	-	Serum	[80]
SARS-CoV	IgM and IgG antibodies	WB	-	1:100 dilution	Serum	[78]
		IFA	1 h 30 min [for IgM] and 30 min [for IgG]	1:10 dilution		
SARS-CoV	IgM, IgG, and IgA antibodies	ELISA	1 h	1:50 dilution	Serum	[50]
		WB	2 h	1:200 dilution		
	IgG antibody	IFA	-	1:40 dilution		
SARS-CoV	IgG antibody	ELISA	1 h 20 min	1:1000 dilution	Serum	[70]
SARS-CoV	IgM, IgG, and IgA antibodies	WB	2-4 h	-	Serum	[51]
		IFA	3 h	1:160 dilution		
SARS-CoV	IgG antibody	ELISA	3 h 30 min	1:200 dilution	Serum	[61]
		WB	-	1:500 dilution		
SARS-CoV	IgM, IgG, and IgA antibodies	ELISA	3 h 15 min	1:10 dilution [for IgM and IgA] and 1:40 dilution [for IgG]	Serum	[18]
		IFA	1 h	1:10 dilution		
SARS-CoV	IgM, IgG, and IgA antibodies	ELISA	2 h 30 min	1:1600 dilution	Serum	[52]
		IFA	1 h 10 min	1:800 dilution		
	Anti-N protein antibody	LFIA	-	-		
SARS-CoV	IgG antibody	ELISA	~ 1 h	1:2000 dilution	Serum	[62]
SARS-CoV HCoV-229E HCoV-OC43	IgG antibody	ELISA	-	-	Serum	[71]
SARS-CoV HCoV-229E HCoV-OC43		WB				
SARS-CoV HCoV-229E HCoV-OC43	IgG antibody	IFA				
SARS-CoV	IgG antibody	IFA	1 h 20 min	1:100 dilution	Serum	[87]
SARS-CoV	IgG antibody	ELISA	1 h 45 min	1:1280 dilution	Serum	[79]
		WB	2 h	1:100 dilution		
SARS-CoV	IgM and IgG antibodies [against S protein]	ELISA	3 h 15 min	1:20 dilution	Serum	[63]
	IgM and IgG antibodies [against N protein]		-	-		
SARS-CoV	IgG antibody	WB	5 h	1:1500 dilution	Serum	[57]
SARS-CoV HCoV-229E	IgM and IgG antibodies	IFA	-	1:800 dilution	Serum	[84]

Table 1 (continued)

Virus	Analyte	Detection method	Assay time	Limit of detection	Tested sample	Reference
SARS-CoV	IgM and IgG antibodies	IFA	2 h	1:160 dilution	Serum	[85]
MERS-CoV				1:20480 dilution		
MERS-CoV	IgG antibody	ELISA	2 h 30 min	1:512 dilution	Camel serum	[64]
MERS-CoV	Anti-N protein antibody	IFA	6 min	200 µg/ml	Spiked antibody solutions	[59]
SARS-CoV-2	IgM, IgG, and IgA antibodies	ELISA	3 h 10 min- 4 h 10 min	-	Serum and plasma	[53]
SARS-CoV-2	IgM and IgG antibodies	CLIA	~ 30 min	-	Serum	[92]
SARS-CoV-2	IgG antibody	IFA	~ 15-19 h	1:100 dilution	Serum	[54]
SARS-CoV-2	IgM and IgG antibodies	ELISA	~ 1 h 45 min	1:100 dilution	Serum	[55]
		CLIA	23 min	-		
SARS-CoV-2	IgM and IgG antibodies	ELISA	1 h 45 min	1:100 dilution	Serum	[44]
SARS-CoV-2	IgG antibody	ELISA	~ 1 h	1:100 dilution	Serum	[100]
SARS-CoV-2	IgM and IgG antibodies	ELISA	3 h 15 min	1:100 dilution	Serum and plasma	[101]
SARS-CoV-2	IgM and IgG antibodies	CLIA	-	-	Serum	[93]
SARS-CoV-2	IgM and IgG antibodies	ELISA	1 h 45 min	-	Serum	[58]
		LFIA	10 min		Plasma	
SARS-CoV-2	IgM and IgG antibodies	ELISA	1 h 15 min	1:800 dilution [for IgM] and 1:80 dilution [for IgG]	Serum	[56]
		LFIA	5-10 min	1:160 dilution		
SARS-CoV-2	IgM and IgG antibodies	ELISA	-	-	Plasma	[65]
	IgG antibody					
	IgM antibody					
Biosensors						
SARS-CoV	Anti-E protein antibody	Optical (SPR)	10 min	200 ng/ml	Spiked antibody solutions	[16]
SARS-CoV	Anti-E protein antibody	Optical (SPR)	1 h	-	Spiked antibody solutions	[17]

HCoV human coronavirus, *SARS-CoV* severe acute respiratory syndrome-related coronavirus, *MERS-CoV* Middle East respiratory syndrome-associated coronavirus, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *IgA* immunoglobulin A, *N* nucleocapsid, *S* spike, *E* envelope, *IFA* immunofluorescence assay, *ELISA* enzyme-linked immunosorbent assay, *LFIA* lateral flow immunoassay, *WB* western blot, *CLIA* chemiluminescence immunoassay, *SPR* surface plasmon resonance

at a serum dilution of 1:20. This assay had a sensitivity of 98.6% and 93.9% for IgG and IgM antibodies, respectively. The N-protein-based ELISA had a sensitivity of 94.7% and 55.2% for the respective antibodies [18, 63]. Furthermore, during that year, Che et al. developed an ELISA platform that exploited N proteins as the antigens and detected IgG antibodies against SARS-CoV, HCoV-OC43, and -229E in serum specimens [71].

In the field of MERS diagnosis, a study was performed by Fukushi and coworkers in 2018. They produced a labeled monoclonal IgG antibody against the S protein of MERS-CoV and used it to establish a competitive ELISA. The sensitivity and specificity of the assay for Ethiopian dromedary camel serum samples were 98% and 100%, respectively. The test took about 2 hours and 30 minutes to complete. This

competitive ELISA was able to detect the target antibodies in 1:512-diluted camel sera [64].

Continuous efforts are still in progress to develop highly sensitive serological ELISA platforms for anti-SARS-CoV-2 detection and COVID-19 diagnosis. Amanat et al. proposed a highly sensitive and specific ELISA for the detection of IgG, IgM, and IgA antibodies in patients' serum or plasma samples. Recombinant S proteins were employed as the capture molecules. The assay took approximately 3 to 4 hours, depending on whether the samples were activated by heat treatment [53]. Lin et al. and Liu et al. designed two different 1-hour-and-45-minute ELISA-based tests to detect IgG and IgM antibodies in 1:100-diluted sera [44, 55]. Both of these assays utilized N proteins as the immobilized capture antigens. A sensitivity of 81.5% was observed for the assay developed by Liu et al.
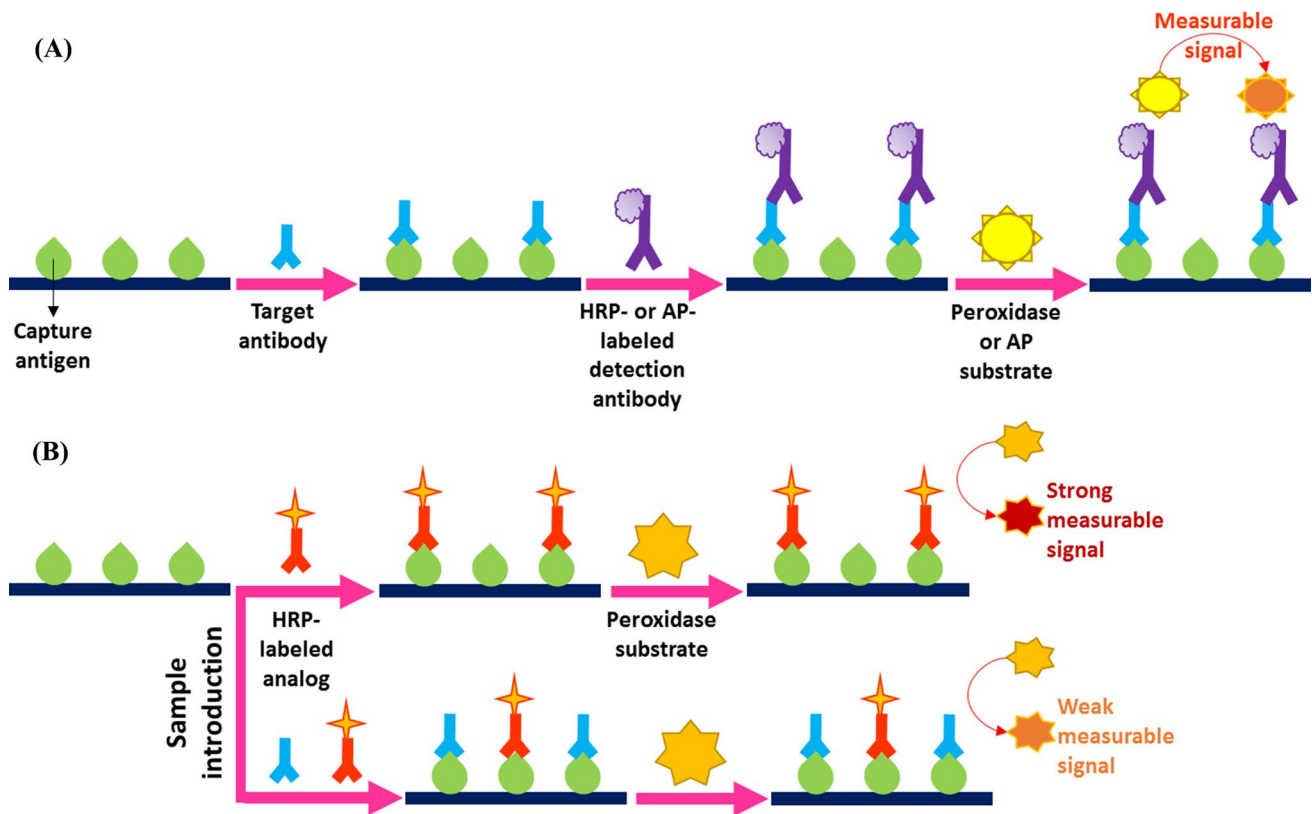
(A)

(B)

Fig. 1 Schematic representations of (**A**) indirect and (**B**) competitive ELISA techniques

In another study, Xiang et al. developed two ELISA-based serological tests for the detection of IgG and IgM antibodies in serum samples. Each assay took 1 hour and 45 minutes. The IgG- and IgM-specific tests employed recombinant SARS-CoV-2 antigens and anti-IgM monoclonal antibodies as the capture molecules, respectively. The sensitivity of the IgG and IgM assays were 87.3%, with a specificity of 100% [58]. Zhang et al. reported a 1-hour-and-15-minute ELISA for the detection of IgG and IgM antibodies in 1:80 and 1:800 serum dilutions, respectively. They investigated the use of three prokaryotically expressed N proteins as well as three eukaryotically expressed S proteins as the capture antigens and concluded that the latter proteins were more appropriate for anti-SARS-CoV-2 serology [56].

Western blot (WB)

The WB method, also known as immunoblotting, is a common laboratory technique applied to separate and identify specific proteins among a pool of protein molecules derived from a particular tissue. This method is also used to examine protein expression [72–74]. Furthermore, this technique has been employed in developing serological assays to detect antiviral antibodies in patients' sera, e.g., WB-based

serological tests for human immunodeficiency virus (HIV), influenza A and B viruses, and CoVs [57, 75, 76].

A WB assay typically consists of three steps; (1) protein separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), (2) transfer of the separated proteins to a nitrocellulose (NC) or polyvinylidene difluoride (PVDF) membrane, and (3) staining and identification of the proteins using an indirect detection assay. In step one, the negatively charged sodium dodecyl sulfate (SDS) renders the proteins as particles with anionic properties. During electrophoresis, depending on their molecular weight and electric charge, the proteins start moving toward the anode through the polyacrylamide gel, which results in their separation (Fig. 2A). As illustrated in Fig. 2B, in the second step, the separated proteins are transferred to an NC or PVDF membrane by exploiting an electric current that moves the anionic proteins toward the anode; hence, the membrane. In step three, as shown in Fig. 2C, the proteins are detected using an indirect assay, which virtually resembles an indirect ELISA. This assay is performed on the membrane that bears the target molecules [74, 77]. In WB-based serological assays for CoVs, anti-CoV antibodies are first captured by CoV-specific N or S proteins and HRP- or AP-labeled detection antibodies recognizing the antigen-antibody complexes are then added. Finally, an
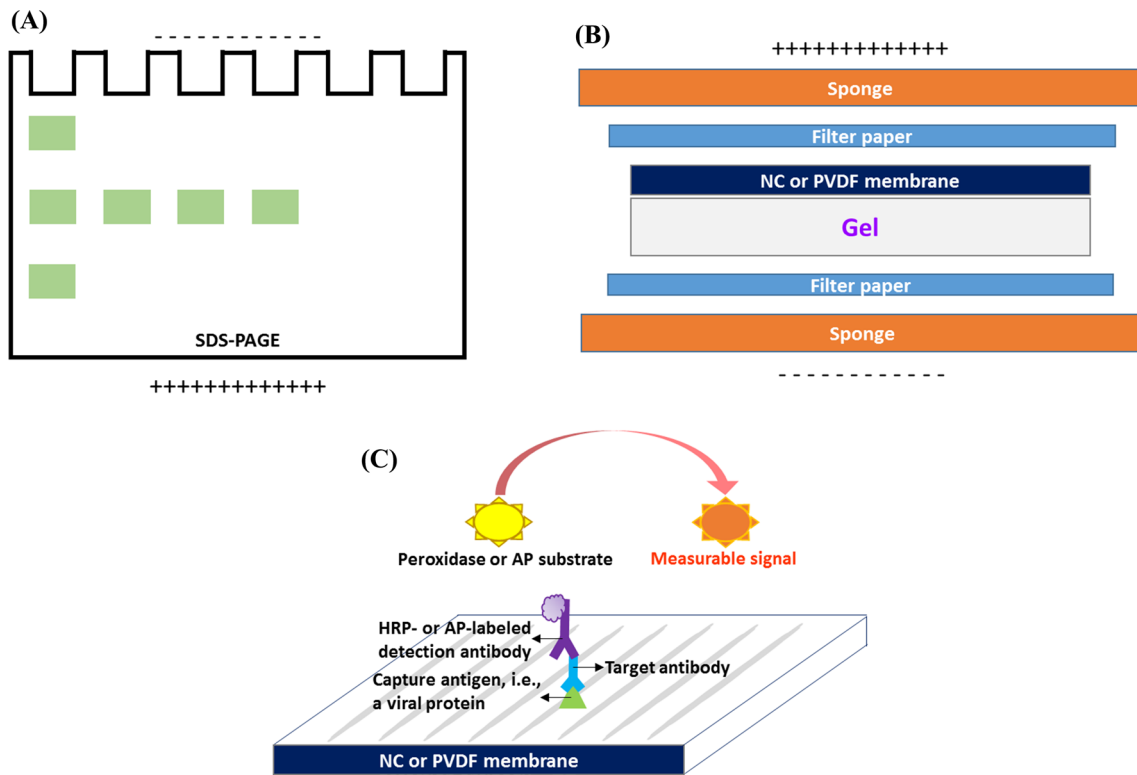
Fig. 2 WB-based serological assays are performed in three steps. (**A**) In the first step, the proteins are separated using SDS-PAGE. (**B**) In the second step, the separated proteins are transferred to an NC or PVDF membrane. (**C**) In the third step, the proteins are identified using an indirect detection assay.

appropriate peroxidase or phosphatase substrate is added to produce a measurable signal, such as a light or color change. The peroxidase substrates employed in CoV-specific WB assays include enhanced chemiluminescence (ECL) and 3, 3'-diaminobenzidine tetrahydrochloride (DAB) [50, 78]. 5-Bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT) is an AP substrate that is used in these assays [61, 79, 80].

From 2004 to 2006, eight WB assays for SARS diagnosis were reported; one detected both IgG and IgM antibodies, two detected IgG, IgM, and IgA antibodies, and the other five were specific to IgG. One of these IgG detection assays, which was proposed by Che et al. in 2005, could also detect anti-HCoV-OC43 and -229E antibodies [71]. The time required for these WB assays ranged from 2 to 5 hours [51, 57].

In 2004, He and coworkers reported a WB assay for the detection of IgG and IgM antibodies against SARS-CoV in 1:100-diluted sera. Immunoblotted N proteins on an NC membrane were used as capture antigens. The assay had a sensitivity of 90.9% and a specificity of 98.3% [78]. Guan et al. developed a 2-hour-and-30-minute WB-based serological test that detected IgG antibodies in serum samples to diagnose SARS. Their assay, which had a sensitivity of 100%, utilized an NC platform with N proteins as the capture molecules [80]. Leung and colleagues developed a WB assay for the detection of IgG, IgM, and IgA antibodies against SARS-CoV in 1:200 serum dilutions. The test took 2 hours and used gel-purified N and S proteins on a PVDF membrane as bioreceptors. It was observed that IgG antibodies secreted by SARS patients reacted more strongly with N proteins than with S proteins [50].

A year later, Wang et al. reported a WB-based test for the detection of SARS-specific IgG antibodies in 1:100-diluted serum specimens. This 2-hour assay made use of an NC membrane with immunoblotted N and three truncated S proteins named S1, S2, and S3. Their results indicated that the N-, S1-, S2-, and S3-based WB assays had a sensitivity of 100%, 50%, 30%, and 70%, respectively [79]. In 2006, Maache and coworkers developed a 5-hour WB assay for the detection of IgG antibodies in 1:1500-diluted sera of SARS patients. They employed the N proteins, as well as the S1 and S2 subunits of the S proteins, as the capture antigens on an NC membrane. Similar to the results obtained by Leung et al. and Wang et al., this study also revealed that the sensitivity of the N-protein-based assay was higher than that of the S-protein-based ones and showed that this rather high sensitivity could

have been in part due to the fact that N-protein-specific IgG antibodies are generally secreted profusely during the critical and recovery phases of the disease [57].

Immunofluorescence assay (IFA)

The IFA technique is an approved method that detects target antigens or antibodies in a given sample. These assays employ fluorescent dyes, also known as fluorophores, enabling the visualization of the targets using fluorescence microscopy or imaging, or their quantification through measurement of fluorescent light intensity. There are two different types of IFA: direct and indirect. Immunofluorescence serology uses the latter type. As shown in Fig. 3, in indirect IFA-based serological tests, initially, the immobilized antigens recognize and capture the target antibodies present in the serum samples. Subsequently, fluorophore-labeled detection antibodies are introduced, which tag the targets and allow for their visualization and quantification [81–83]. IFA-based serological assays for CoV antibodies have utilized fluorescein isothiocyanate (FITC), rhodamine, cyanine (Cy) 3 and 5, DyLight 649, and quantum dot fluorophores as the fluorescent labels [51, 54, 59, 84, 85].

As shown in Table 1, from 2003 to 2020, there were fifteen studies developing IFA-based serological tests as a means to diagnose SARS, MERS, COVID-19, HCoV-OC43, and HCoV-229E. Four of the assays were applicable for the identification of IgG, IgM, and IgA antibodies, three of them detected the IgG and IgM antibodies, and the others were specific to IgG antibodies only. The time required for these assays varied from 6 minutes to almost 19 hours [54, 59].

In 2004, Chan et al. described an IFA for the detection of SARS-CoV-specific IgG antibodies in 1:40 serum dilutions. The assay, which employed whole virions as the immobilized capture agents, took 1 hour and 30 minutes and had a sensitivity and specificity of 100% [86]. In addition to their WB assay, He and coworkers reported an IFA-based test in which they used whole virus particles as the capture antigens. The assay detected IgG and IgM antibodies in 1:10-diluted sera from SARS patients within 30 and 90

minutes, respectively [78]. Moreover, Wu et al. designed an IFA for the detection of SARS-CoV that identified the IgG, IgM, and IgA antibodies in 1:800 serum dilutions within 1 hour and 10 minutes. This assay utilized virus-infected cells as the source of whole virus antigens and had a sensitivity and specificity of 99.1% and 87.8%, respectively [52].

A year later, in addition to their ELISA and WB tests, Che et al. developed an IFA-based serological test for the detection of IgG antibodies against SARS-CoV, HCoV-OC43, and HCoV-229E in human sera. The assay employed whole virus molecules as capture antigens [71]. Manopo and coworkers described an S protein-based IFA for the detection of SARS-specific IgG antibodies in 1:100 serum dilutions. The 1-hour-and-20-minute assay had 100% sensitivity and specificity [87]. In 2006, Zhu et al. reported an IFA-based test for the detection of IgG and IgM antibodies in 1:800-diluted sera from SARS and HCoV-229E patients. The assay made use of N proteins as the capture molecules [84].

In 2013, Reusken et al. developed an IFA assay for the detection of IgG and IgM antibodies against both SARS- and MERS-CoV in human serum samples. The 2-hour assay exploited the S1 subunit's receptor-binding domains (RBDs) to capture the target antibodies, and detected them in 1:160- and 1:20480-diluted sera for SARS- and MERS-CoV, respectively [85]. Additionally, in 2019, Hoy and colleagues designed an IFA-based serological test that used electrospun polystyrene microfibers as three-dimensional membrane filters and FITC as the fluorescent label for the detection of anti-N protein antibodies against MERS-CoV. This 6-minute assay was able to simultaneously detect anti-human serum albumin antibodies (anti-HSA), as well as the anti-MERS-CoV antibodies. The detection limit for the anti-N protein antibodies in the spiked antibody solutions was 200 μg/ml [59].

A year later, in 2020, during the ongoing COVID-19 pandemic, Khan and coworkers described an IFA-based protein microarray composed of 67 antigens on an NC-coated slide to examine the cross-reactivity between the previous CoVs and the latest one, SARS-CoV-2. This assay examined the seroreactivity of IgG antibodies in 1:100 serum dilutions
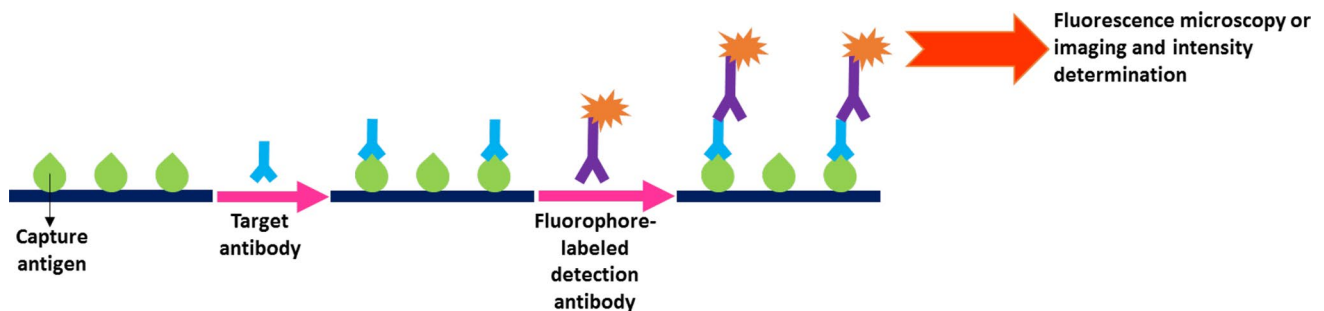

Fig. 3 A schematic illustration of the indirect IFA exploited in CoV-specific serology

toward the CoVs. The assay time was roughly 15 to 19 hours, since it involved the overnight incubation of sera. The results demonstrated that despite the high reactivity of the IgG antibodies to the previous CoVs, these antibodies showed low seroreactivity to SARS-CoV-2, with some reactivity to the S2 subunit and the N protein [54].

Lateral flow immunoassay (LFIA)

LFIA, also known as the immunochromatographic test (ICT), is a rapid paper-based assay capable of determining the presence of an analyte in a given sample within a time frame of only 5 to 30 minutes. The applications of LFIA-based tests include food and environment quality assessments as well as medical diagnosis. Test strips have been utilized to analyze samples of whole blood, plasma, serum, urine, sweat, or saliva as body fluids specimens. The assay's platform is a strip consisting of an inert backing on which four overlapping zones have been assembled using a pressure-sensitive adhesive. These zones are (1) the sample, and (2) the conjugate pads, (3) the NC membrane, and (4) the absorbent pad [88, 89].

As illustrated in Fig. 4, in LFIA-based serological tests, the sample is first dropped onto the sample pad and then channeled into the next zone, i.e., the conjugate pad, through capillary flow. This pad commonly contains tagged capture antigens or anti-antibodies specific to the target molecules. The labels can be colloidal gold, monodisperse latex, colloidal carbon, fluorophores, or quantum dots. For instance, the use of colloidal gold enables the visualization of a red product by the naked eye. After the interaction between the targets and the capture molecules, the resulting complexes flow to the NC membrane, comprising the test and control lines, on which detection antigens and control antigens or antibodies have been immobilized, respectively. Lastly, the absorbent or wick pad absorbs the processed sample through the capillary effect, thus preventing it from flowing back into the NC membrane and the conjugate pad. Generally, the test result is positive when both of these lines adopt a color visible by the naked eye or observable using a fluorescence analyzer. If the color is seen only on the control line, it signifies a negative result, and if the color appears only on the test line, it indicates that the test is inaccurate and was not appropriately implemented [88–90].
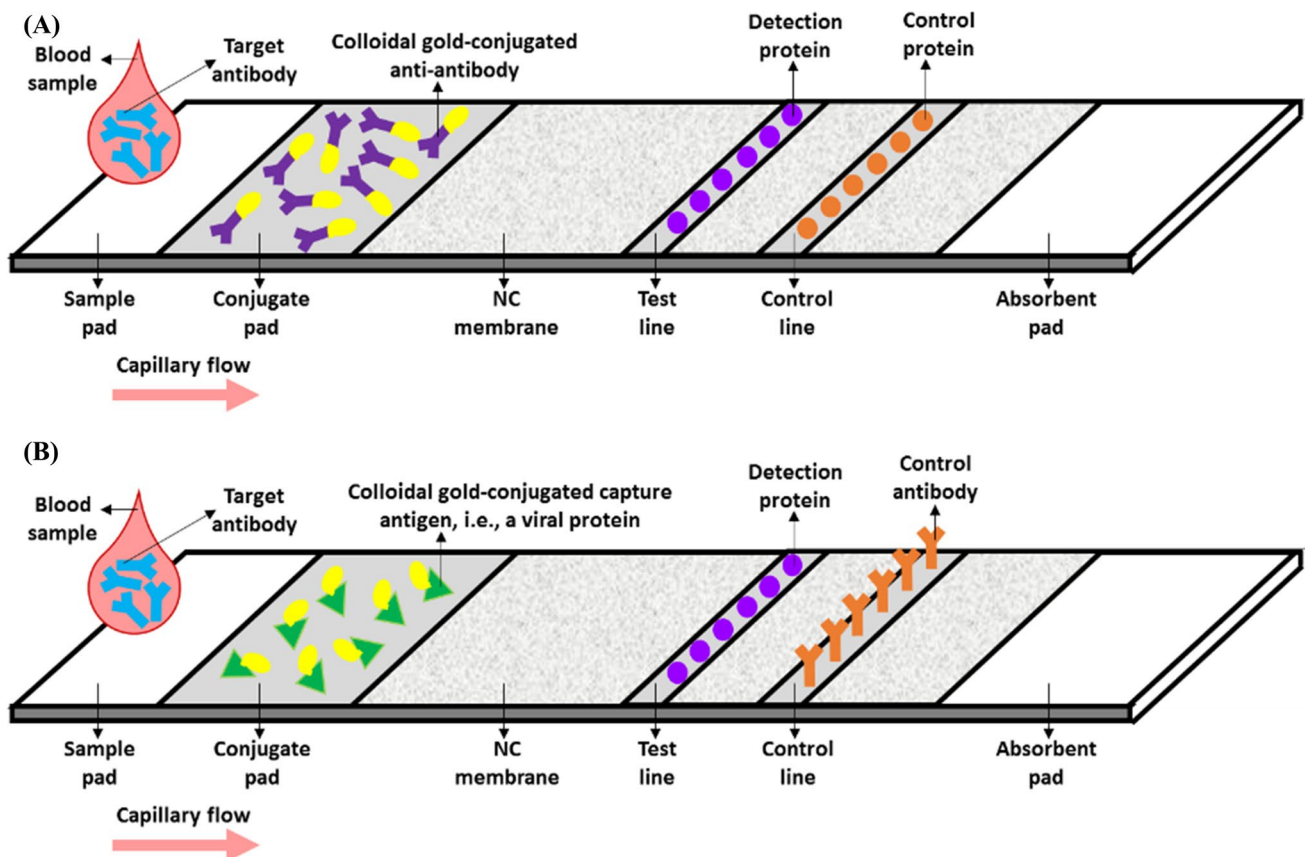

Fig. 4 (**A** and **B**) Schematic illustration of two potential LFIA-based serological assays for CoV detection

In 2004, in addition to their ELISA-based tests discussed in section 2-1, Guan et al. developed two LFIAs that detected the IgG antibodies against SARS-CoV in undiluted sera within 15 minutes. Both assays employed colloidal gold-tagged anti-IgG antibodies, N proteins, and proteins unique to SARS-CoV as the capture, detection, and control agents, respectively (Fig. 4A). A commercial IFA was used to validate these tests, which were found to have a sensitivity and specificity of 70.5-100% and 97.7-100%, respectively [68, 69]. Wu et al. described an LFIA for the detection of anti-N-protein antibodies in the sera of SARS patients. The assay's conjugate pad contained colloidal gold-conjugated N proteins and mouse IgG antibodies. N proteins and anti-mouse IgG antibodies were immobilized onto the test and control lines, respectively. The assay had a sensitivity of 33.6% and a specificity of 98.2% [52].

In 2020, as a means to identify COVID-19 patients, Xiang et al. reported an ICT that was employed to detect the IgG and IgM antibodies associated with SARS-CoV-2 in the patients' plasma specimens. The 10-minute assay had a sensitivity and specificity of 82.4% and 100%, respectively [58]. Zhang et al. designed an LFIA for the detection of IgG and IgM antibodies in 1:160-diluted sera of COVID-19 patients within 5 to 10 minutes. This test made use of colloidal gold-labeled RBDs of recombinant S proteins, recombinant S1 subunits, and secondary polyclonal antibodies as the capture, detection, and control probes, respectively (Fig. 4B). The sensitivity of the assay was 86.89%, and its specificity was 99.39% [56].

Chemiluminescence immunoassay (CLIA)

CLIA is a rapid and ultrasensitive immunoassay that is widely applied for the detection of biological and environmental molecules, such as proteins, nucleic acids, hormones, and pollutants. This label-based technique employs either chemiluminescent tags such as luminol and isoluminol or enzymatic labels such as HRP or AP, followed by the addition of a luminol-based substrate that generates a light signal, the intensity of which can be measured using a chemiluminescent analyzer [91]. Fig. 5 illustrates a CLIA-based serological test utilizing enzymatic tags with their specific chemiluminescent substrates.

In addition to their ELISA-based serological test, Lin and coworkers developed a CLIA using magnetic bead-conjugated recombinant N proteins, AP-labeled anti-immunoglobulin antibodies, and Lumigen APS-5 as the capture agents, detection probes, and the AP-specific chemiluminescent substrate, respectively. This assay detected IgG and IgM antibodies against SARS-CoV-2 in undiluted serum samples within 23 minutes. The test had a sensitivity of 82.28% for both antibody types, and a specificity of 97.5% and 81.25% for IgG and IgM antibodies, respectively [55].

Cai and colleagues designed a magnetic CLIA to detect IgG and IgM antibodies in the sera of COVID-19 patients. This assay, which took approximately 30 minutes, was based on magnetic-bead-bound peptides derived from the S, N, or open reading frame 1a and 1b (ORF1a/b) proteins specific to SARS-CoV-2. Their results indicated that the CLIA employing the S proteins as the capture antigens demonstrated the best performance, with a positive detection rate of 71.4% and 57.2% for IgG and IgM antibodies, respectively [92]. Qu et al. described a CLIA-based serological test that identified IgG and IgM antibodies against SARS-CoV-2 in serum specimens. They employed S and N proteins to heighten the assay's sensitivity. The assay had a specificity of 95% for both types of antibodies and a sensitivity of 90% and 80% for IgG and IgM antibodies, respectively [93].
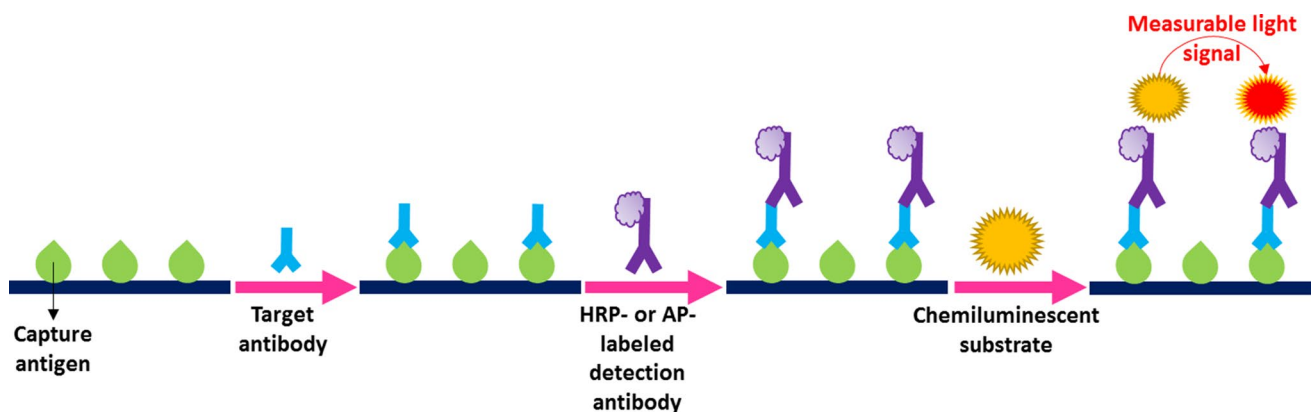

Fig. 5 Schematic illustration of a potential CLIA-based serological test for CoV-associated disease diagnosis

Biosensors

Biosensors offer features such as rapid analysis, high sensitivity and specificity, and cost-effectiveness. Therefore, they are promising candidates for the detection of pathogenic particles. To date, a variety of electrochemical, electrical, optical, piezoelectric, and mechanical biosensors have been reported as media for virus detection [94–96].

Regarding SARS-CoV-specific serology, two label-free SPR-based optical biosensors were reported by Park et al. in 2009 and 2011. In the first study, they developed an SPR-based assay for the rapid detection of anti-SARS-CoV E protein antibodies. They fabricated a gold micro-patterned chip and immobilized the E proteins on the gold surface through gold binding polypeptides. After SPR imaging analysis, the biosensor identified as little as 200 ng of the target antibodies per ml within 10 minutes in spiked antibody solutions (Fig. 6A). In their other work, they presented an SPR-like reflective biosensor for anti-E protein antibody detection. They exploited functionalized colloidal silica photonic crystals and the SPR-like characteristics of their reflectance spectra and detected SARS-CoV-associated antibodies within an hour (Fig. 6B) [16, 17].

Commercial serological assays for COVID-19 diagnosis

Since the COVID-19 pandemic, a multitude of serology-based tests have been under development, and several have reached the commercialization status [102]. Table 2 lists various commercialized assays that have received United States Food and Drug Association (FDA) Emergency Use Authorization (EUA) or European CE approval. These assays are ELISA-, IFA-, LFIA, or CLIA-based. As shown in Table 2, the ELISA-, LFIA-, and CLIA-based tests have comparatively high sensitivity and specificity. Due to the urgent demand for COVID-19 screening worldwide, LFIA-based serological testing seems to be favored due to its

high sensitivity and specificity, rapid response, and easy application. It is less operator-dependent than other assays, which even if automated, still require sophisticated equipment. Moreover, because COVID-19 is often asymptomatic and the level of IgM antibodies tends to decline within 14 days of the disease onset, a rapid diagnostic assay, e.g., an LFIA, detecting IgG and IgM antibodies simultaneously is recommended.

Concluding remarks and future perspectives

The conventional serological assays, including ELISA, WB, IFA, LFIA, and CLIA, as well as biosensors as platforms for the detection of antibodies against HCoVs in human whole blood, serum, or plasma samples, have been discussed in this review. During the SARS, MERS, and COVID-19 outbreaks, these tests have been mostly applied alongside NAATs as rapid and inexpensive alternatives for large-scale screening. Despite the statement of WHO on April 24, 2020, regarding the most recently identified HCoV, SARS-CoV-2, these assays have been applied for analyzing the transmission of CoVs and identifying newly infected and recovered patients who have potentially become immune to the disease. The sensitivity of serological assays varies among patients and is profoundly dependent on the time of the infection; since the amount of anti-CoV antibodies secreted generally depends on the time since viral exposure.

In conclusion, further research is required to understand the effect of the host antibodies on SARS-CoV-2; in order to determine whether these assays can be used to identify individuals who have become immune to the disease. Nonetheless, their rapid response and low cost have made serological tests attractive candidates for screening large numbers of people with suspected infection, in contrast to the time-consuming and costly NAATs, such as RT-PCR assays. Among the conventional ELISA-, IFA-, LFIA-, and CLIA-based serological tests, an LFIA identifying both IgG and IgM antibodies seems to be a desirable choice due to its relatively high sensitivity and specificity and rapid response,
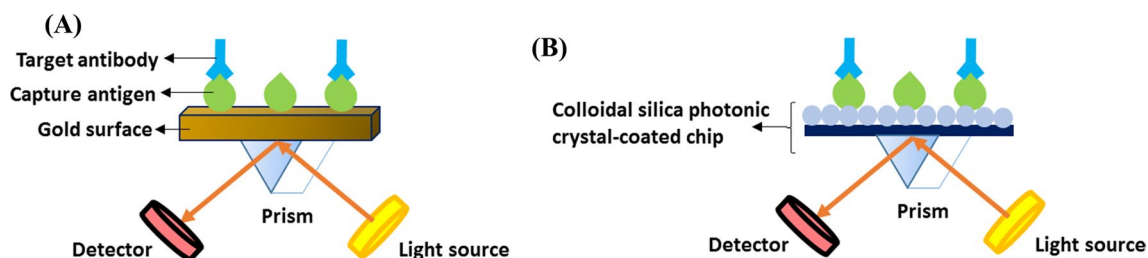

Fig. 6 Schematic illustrations of two optical biosensors for SARS-CoV-specific antibody detection. (**A**) The first one is an SPR-based biosensor employing E proteins immobilized on a gold surface as the capture probes. (**B**) The second biosensor is an SPR-like reflective platform utilizing the same proteins attached to a colloidal silica photonic crystal-coated chip

Table 2 Commercialized anti-SARS-CoV-2 detection assays

Product name	Manufacturer	Regulatory approval	Target antibody/ capture molecule	Assay time	Sensitivity/ specificity	Tested sample	Reference(s)
ELISA-based tests							
Anti-SARS-CoV-2 ELISA (IgG)	EUROIMMUN AG, Lübeck, Germany	FDA EUA	IgG/S1 subunit of the S protein	2 h 36 min	90%/100%	Serum or plasma	[103]
SCoV-2 Detect™ IgG ELISA	InBios International, Inc., Seattle, Washington, United States	FDA EUA	IgG/SARS-CoV-2 antigen	2 h 3 min	100%/100%	Serum	[104]
WANTAI SARS-CoV-2 Ab ELISA	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China	FDA EUA	IgG/recombinant RBD of the S protein; IgM/recombinant RBD of the S protein	1 h 35 min	96.7%/97.5%	Serum or plasma	[105]
Platelia SARS-CoV-2 Total Ab	Bio-Rad Laboratories, Inc., Hercules, California, United States	FDA EUA	IgG/recombinant N protein; IgM/recombinant N protein; IgA/recombinant N protein	2 h 5 min	98%/99.33%	Serum or plasma	[106]
IFA-based tests							
Fosun Novel Coronavirus (2019-nCoV) IgM/IgG Test Kit	Shanghai Fosun Long March Medical Science Co., Ltd., Shanghai, China	CE	IgG/-; IgM/-	~ 15 min	36.7%/ 98.8%; 10%/96.2%	Serum or plasma	[107, 108]
POCT-fluorescence Coronavirus IgG/IgM antibodies detection kit	Sichuan Xincheng Biological Co., Ltd., Chengdu, Sichuan, China	CE	IgG/recombinant N protein and RBD of the S protein; IgM/recombinant N protein and RBD of the S protein	-	93%/100%; 84%/100%	Whole blood, serum, or plasma	[109]
LFIA-based tests							
Cellex qSARS-CoV-2 IgG/IgM Rapid Test	Cellex, Inc., Durham, North Carolina, United States	FDA EUA	IgG/recombinant N and S proteins; IgM/recombinant N and S proteins	15-20 min	93.75%-96.4%	Whole blood, serum, or plasma	[110]
COVID-19 IgG/IgM Rapid Test Cassette	Healgen Scientific, LLC., Houston, Texas, United States	FDA EUA	IgG/recombinant S1 subunit of the S protein; IgM/recombinant S1 subunit of the S protein	10-15 min	96.7%/98%; 86.7%/99%	Whole blood, serum, or plasma	[111]
Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	Biohit Healthcare (Hefei) Co., Ltd., Hefei, China	FDA EUA	IgG/recombinant N protein; IgM/recombinant N protein	15-20 min	96.7%/95%	Whole blood, serum, or plasma	[112]
LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit (Colloidal Gold)	Hangzhou Laihe Biotech Co., Ltd., Hangzhou, China	FDA EUA	IgG/recombinant S1 subunit of the S protein; IgM/recombinant S1 subunit of the S protein	10-15 min	100%/98.8%	Serum or plasma	[113]
RightSign™ COVID-19 IgG/ IgM Rapid Test Cassette	Hangzhou Biotest Biotech Co., Ltd., Hangzhou, China	FDA EUA	IgG/recombinant RBD of the S protein; IgM/recombinant RBD of the S protein	10-20 min	93.3%/100%; 100%/100%	Whole blood, serum, or plasma	[114]

Table 2 (continued)

Product name	Manufacturer	Regulatory approval	Target antibody/capture molecule	Assay time	Sensitivity/specificity	Tested sample	Reference(s)
CLIA-based tests							
LIAISON® SARS-CoV-2 S1/S2 IgG	DiaSorin, Inc., Saluggia, Italy	FDA EUA	IgG/recombinant S1 and S2 subunits of the S protein	35 min	94.4%/97.8%	Serum or plasma	[115]
SARS-CoV-2 IgG (CoV-2 IgG)	Abbott Laboratories, Chicago, Illinois, United States	FDA EUA	IgG/recombinant SARS-CoV-2 antigen	-	96.77%/99.63%	Serum or plasma	[116]
Atellica IM SARS-CoV-2 Total (COV2T)	Siemens Healthineers, Erlangen, Germany	FDA EUA	IgG/recombinant RBD of the S protein; IgM/recombinant RBD of the S protein	~10 min	100%/99.82%	Serum or plasma	[117]
VITROS® Immunodiagnostic Products; Anti-SARS-CoV-2 Total Reagent Pack Test	Ortho Clinical Diagnostics, Inc., Raritan, New Jersey, United States	FDA EUA	IgG/recombinant S1 subunit of the S protein; IgM/recombinant S1 subunit of the S protein; IgA/recombinant S1 subunit of the S protein	~48 min	100%/100%	Serum or plasma	[118]
Elecsys® Anti-SARS-CoV-2 immunoassay	Roche Diagnostics, Basel, Switzerland	FDA EUA	IgG/recombinant N protein; IgM/recombinant N protein; IgA/recombinant N protein	~18 min	99.5%/99.8%	Serum or plasma	[119, 120]

FDA Food and Drug Association, *EUA* Emergency Use Authorization, *SARS-CoV-2* severe acute respiratory syndrome-related coronavirus-2, *COVID-19* CoV disease 2019, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *IgA* immunoglobulin A, *N* nucleocapsid, *S* spike, *RBD* receptor-binding domain, *ELISA* enzyme-linked immunosorbent assay, *IFA* immunofluorescence assay, *LFIA* lateral flow immunoassay, *CLIA* chemiluminescence immunoassay, *POCT* point-of-care testing

which satisfies the urgent need for COVID-19 screening and a simple testing procedure.

Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing interests or personal relationships that could have influenced the work reported in this article.

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