Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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A review post-vaccination SARS-CoV-2 serological test: Method and antibody titer response

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ABSTRACT

The development of the Coronavirus disease 2019 (COVID-19) vaccine is one of the most important efforts in controlling the pandemic. Serological tests are used to identify highly reactive human donors for convalescent plasma therapy, measuring vaccine efficacy and durability. This review article presents a review of serology tests and how antibody titers in response to vaccines have been developed. Some of the serological test methods discussed are Plaque Reduction Neutralization Test (PRNT), Enzyme-Linked Immunosorbent Assay (ELISA), Lateral flow immunoassay (LFIA), chemiluminescent immunoassay (CLIA), and Chemiluminescent Micro-particle Immunoassay (CMIA). This review can provide an understanding of the application of the body's immune response to vaccines to get some new strategies for vaccines.

1. Introduction

On December 31, 2019, WHO China Office announced that a case of pneumonia of unknown etiology was detected in Wuhan City, China. This virus spreads quickly and is transmitted from human to human through droplets. In February 2020, the virus was defined as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease was named coronavirus disease 2019 (COVID-19). On March 13, the WHO reported COVID-19 as a global pandemic which is continuing today. As of August 23, 2022, more than 590 million cases of infection with 6 million deaths have been reported worldwide [1].

Various countermeasures have been taken to address this event including accelerating the development, manufacture, and deployment of a vaccine against COVID-19 [2]. The development of vaccines is considered one of the most important efforts in controlling the pandemic by forming the immune system to reduce the risk of death and severe symptoms due to COVID-19. By the end of 2020, several vaccines were ready for use in various parts of the world with emergency approval. To date, several vaccines have been licensed for use (Table 1) and around 12 billion doses of vaccine have been distributed worldwide [1].

Although vaccination has been carried out by most countries, data on how protective and how long the antibodies will last are not clear. To better understand the human body's immune response to vaccines, serological tests are very important. Serological testing is a technique of measuring an individual's antibody levels using a blood sample [5]. Serological tests for SARS-CoV-2 specific antibodies can determine whether vaccination results in the production of SARS-CoV-2 specific antibodies or not [6]. In addition, serological tests for SARS-CoV-2 were used to identify highly reactive human donors for convalescent plasma therapy, measuring the efficacy and durability of the vaccine [7]. Antibody tests are more suitable for public health surveillance, vaccine development, and follow-up vaccinations than for diagnosis [8].

Serological tests have different uses from PCR tests. The PCR test is used as a confirmatory test for COVID-19 when a person has the virus in their respiratory secretions. This is useful in settings with a high incidence of active infection, symptomatic patients, and contact tracing. Meanwhile, serological tests are used to answer questions about whether an individual has an immune response to the COVID-19 virus, how long the antibodies last, whether individuals suffering from COVID-19 need a vaccine, which vaccine is better, and when to re-vaccinate [7].

Serological tests can be broadly categorized based on the reading platform used to test for SARS-CoV-2 antibodies [5]. Several serologic test methods include the plaque reduction neutralization test (PRNT) as the gold standard for measuring antibodies in blood serum [9], Medium-throughput immunoassays such as enzyme-linked immunosorbent assay (ELISA) [6,10,11], and Lateral flow immunoassay (LFIA) [12], and high throughput such as immunofluorescence assay (IFA) [7], chemiluminescent immunoassay (CLIA) [13], Electro-chemiluminescence Immunoassay (ECLIA) [14], and Biosensor [15,16]. This review article aims to provide information related to various

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Table 1
List of vaccines that have received permission to use [3,4].

Vaccine name	Producer	Basic material	Dosage (dose interval)
Ad26.CoV2·S	Janssen/Johnson & Johnson (US)	Virus vector	1 dose
BBIBP-CorV	Sinopharm (China)	Inactivated virus	2 doses (3 weeks)
BBV152 (COVAXIN)	Bharat Biotech (India)	Inactivated antigen	2 doses (4 weeks)
BNT162b2	Pfizer-BioNTech (US)	mRNA	2 doses (3 weeks)
ChAdOx1 (AZS1222)	AstraZeneca/Oxford (UK)	Virus vector	2 doses (4 weeks)
CoronaVac	Sinovac Biotech (China)	Inactivated virus	2 doses (2 weeks)
CVnCoV	CureVac/GlaxoSmithKline (German)	mRNA	2 doses (4 weeks)
Gam-COVID-Vac (Sputnik V)	Gamaleya National Research Center for Epidemiology and Microbiology (Rusia)	Virus vector	2 doses (3 weeks)
mRNA-1273	Moderna (US)	mRNA	2 doses (4 weeks)
NVX-CoV2373	Novavax, Inc (US)	Subunit proteins	2 doses (3 weeks)

SARS-CoV-2 serological test methods and understand the body's immune titers resulting from vaccination.

1.1. Serological test method

A serological test also called an antibody test, is an in-vitro test for antigen-antibody reactions. This test is carried out on blood serum samples to detect antibodies or antigens specifically for certain diseases [17]. Serology is used to follow up on vaccinations and provide data on antibody responses obtained after the first and second vaccinations in various countries [6].

After the vaccine or SARS-CoV-2 infection, our immune system produces antibodies, but not all antibodies can block viral infection. This is because some antibodies bind to viral antigens on epitopes that are not essential for viral infection so these antibodies cannot neutralize the virus. Thus, it is imperative to measure individual neutralizing antibody levels for vaccine clinical trials, research studies, and disease prevention [5].

The accuracy and reliability of the serological method largely depend on the choice of the targeted SARS-CoV-2 antigen and the test format [18]. The main target of neutralizing antibodies against coronavirus is spike protein. Neutralizing antibodies will bind to the RBD S1 protein to block its interaction with angiotensin-converting enzyme 2 (ACE2) and antibodies that bind to other regions can inhibit the S protein conformational change and block membrane fusion [19]. The following are some of the serological test methods.

i. Plaque Reduction Neutralization Test (PRNT)

The most commonly used serological method is the plaque reduction neutralization test (PRNT). PRNT is the "gold standard" method for testing the neutralizing activity of anti-SARS-CoV-2 which requires the patient's serum to be diluted and incubated with the original live virus followed by cell infection [5,20]. The principle of PRNT serological testing is that neutralizing antibodies (NAb) prevent viruses from infecting cells and causing plaque as shown in Fig. 1. PRNT is a method of detecting and measuring antibodies in serum samples by calculating the percentage decrease in viral activity. Antibody titers are conventionally determined by counting the decreased amount of plaque (a localized area of infection due to a cytopathic effect) after mixing the serum sample that has neutralizing antibodies and comparing it with the number of standard viral plaques [21,22].

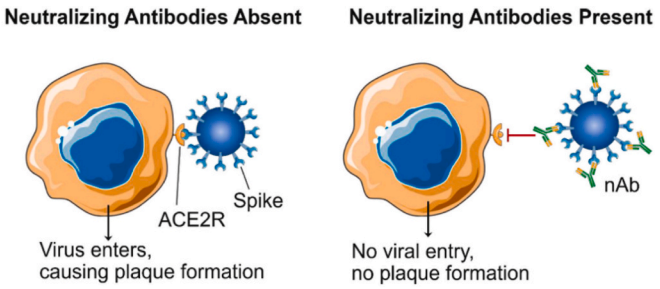


Fig. 1. How the plaque reduction neutralization test (PRNT) works. Reprinted from Ref. [5] with Elsevier's free permission.

The standard used today is PRNT50. PRNT50 is defined as a 50% reduction (50% of Inhibition Concentration or IC50) of the plaque amount after the addition of a serum-containing neutralizing antibody and this is the endpoint of the titration [22].

However, the PRNT test has several drawbacks, making it unsuitable for large-scale testing. PRNT has very low throughput, takes several days (2–4 days), and is biosafety level 3 (BSL3) because it uses live viruses and must be performed by experienced personnel in the laboratory [20,23].

Various methods other than PRNT have been developed to get faster results. However, PRNT is still used as a comparison/standard in several studies. Wisnewksi et al. [9] compare competitive ELISA results with the PRNT test. Kohmer et al. [20] compared Abbott SARS-CoV-2 IgG II Quant test quantity with PRNT to get clinical performance. Muruato et al. [24] reported a fluorescence-based SARS-CoV-2 antibody neutralization test and PRNT was used as a validation assay. Surrogate virus neutralization test (sVNT) from Genscript compared sensitivity, specificity, and cross-reactivity to PRNT [25].

ii. Lateral Flow Assay (LFA)

LFA is a paper-based platform for the detection and quantification of analytes in mixtures. LFA is a Rapid Diagnostic Test (RDT) or the basis of serological testing at the treatment site and only takes 15 min per sample. Biological samples that can be tested using LFA are urine, saliva, sweat, serum, plasma, blood, and other fluids. LFA is categorized into two different types based on the recognition elements used, namely Lateral Flow Immuno Assay (LFIA) using antibodies as the recognition element and Nucleic Acid Lateral Flow Assay (NALFA) [26].

LFIA uses a cassette into which a patient sample is injected and a tape that appears positive or negative for antibody detection. If there are antibodies in the patient's sample, the antibodies will attach to the viral antigen bound to the gold nanoparticles. The complex migrates along the membrane to reach the test line containing secondary antibodies to the immune complex causing a color change detectable by the human eye as shown in Fig. 2. However, the positive/negative band in LFA is often difficult to read and reading training is required for reliable assays [5]. The LFA test is more expensive and time-consuming than large-scale testing [27].

iii. Enzyme-Linked ImmunoSorbent Assay (ELISA)

ELISA is a biochemical method that combines an immunoassay with an enzymatic assay [29]. ELISA uses the basic immunological concept of an antigen-binding to its specific antibody to detect the antibody/antigen in a fluid sample. ELISA uses enzyme-labeled antigens and antibodies to detect biological molecules [30]. Some of the enzymes that are often used are Horseradish peroxidase (HRP), Alkaline phosphatase (AP), and β-D-galactosidase [31].

There are several types of detection using ELISA depending on the purpose, sample, and reagent purity. The following are the main protocols in ELISA (Fig. 3):

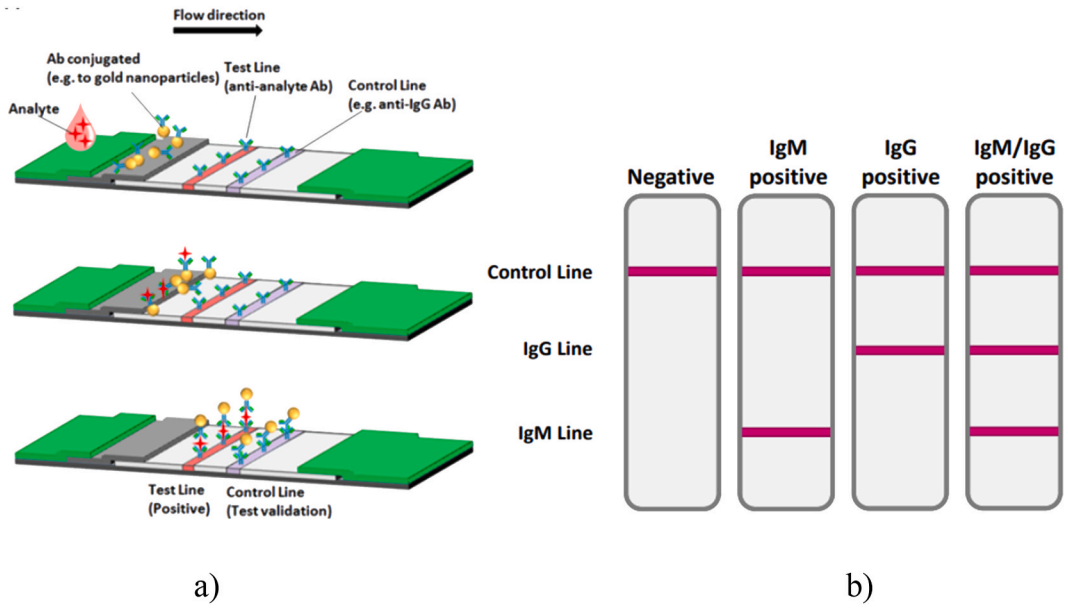


Fig. 2. Lateral Flow ImmunoAssay a) schematic of the test mechanism b) results and interpretation. Reprinted from Refs. [26,28] with Creative Commons license.

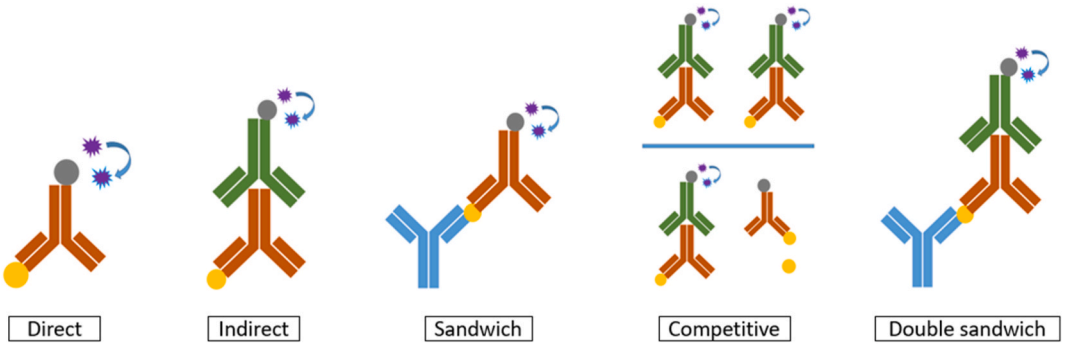


Fig. 3. Types of ELISA testing protocol.

- Direct ELISA

Direct ELISA involves two steps in which the analyte is attached to the plate and the enzyme-labeled primary antibody is introduced. The substrate is then added to the well and the final signal can be recorded. This type of testing generally requires pure isolates to avoid binding from other samples that are not desirable, resulting in test errors. In addition, not all primary antibodies can be labeled with enzymes so this type of testing is limited [31].

- Indirect ELISA

Indirect ELISA has a labeled secondary antibody. This assay provides better accessibility to different types of labeled secondary antibodies, but it has non-specificity [31].

- Sandwich ELISA

The desired analyte is between the primary and secondary antibodies. This strategy provides better specificity because the first biomolecule to be immobilized is the primary antibody which is highly purified. But when the desired analyte does not interact with the primary antibody, the secondary antibody will bind to the primary antibody and produce a false positive signal [31].

- Double sandwich ELISA

This type of test is the most specific ELISA protocol. The analyte of interest is between two antibodies (capture antibody and primary antibody) produced in different host bodies. Therefore, the two antibodies will not bind to each other, and non-specific binding is minimized. After that, the labeled secondary antibody binds to the primary antibody and a detection signal can be recorded. The weakness of this test is the lengthy procedure [31].

- Competitive ELISA

In this test, two sets of experiments were carried out in parallel. The first experiment was carried out by indirect ELISA. In parallel experiments, the primary antibody was incubated with the antigen first. This incubation will give some part of the antibody that is not bound. Furthermore, it is added to the antigen-coated well so that the binding of the primary antibody is reduced because the binding site is already full. The signal received in parallel experiments is inversely correlated with the presence of the desired analyte. The disadvantage of this protocol is that it is a long procedure and consumes a large sample volume but provides high specificity [31].

In several studies, postvaccine SARS-CoV-2 serological assays were carried out according to the principle of competitive binding between the anti-SARS-CoV-2 NAb antibody blocking the enzyme-labeled S-RBD

protein and ACE2 coated on the microtiter plate [20]. The microtiter plate was coated with ACE2 and a sample containing the SARS-CoV-2 neutralizing antibody was added. Next, the RBD S-HRP conjugate was added. Antibodies in the sample will block the binding of the protein with ACE2 (Fig. 4).

The competitive ELISA with ACE2 on the plate experienced consistent technical difficulties due to RBD cross-reaction. As an alternative, a reverse competitive ELISA was performed in which RBD was bound to the plate [9]. Competitiveness occurred between serum sample IgG and soluble ACE2 and was detected by streptavidin-HRP (Fig. 5).

The indirect ELISA protocol was successfully performed and reported [6,32,33]. The RBD S antigen on the ELISA plate was added to the serum sample then IgG labeled HRP was added. Saadat et al. [10] used the indirect ELISA protocol with AP-labeled IgG according to the previous study [34].

The ELISA test can be completed in a few hours in a Biosafety level 1 or 2 environments [12]. The main challenge in implementing ELISA in vaccine development is the selection of appropriate positive control (sample containing analyte) and negative (sample that does not contain analyte, aiming to check for non-specific binding). At the experimental stage of vaccine development and with unknown samples, it is very difficult to achieve high analytical precision [31]. However, the ELISA technique has been widely used for vaccine trials around the world.

iv. Immunofluorescence Assay (IFA)

IFA is a technique based on the antigen-antibody reaction in which antibodies are labeled with a fluorescent dye. The antigen-antibody complex is visualized using an ultraviolet (fluorescent) microscope [35]. Fluorophores are dyes that absorb ultraviolet radiation so that they are excited and emit visible light. The most commonly used fluorophore is fluorescein isothiocyanate (FITC) [36].

The principle of IFA testing is divided into two, namely direct and indirect as shown in Fig. 6. In direct IFA, labeled antibodies react directly with antigens in serum samples, while indirect IFA is based on antibody detection by antigens [37]. Therefore, for the serological test for SARS-CoV-2, the principle of indirect testing is carried out.

ELISA and LFIA have limited value for a single response to an antigen. Alternatively, antigen microarrays with the IFA principle are used to detect antibodies of several isotypes against hundreds of antigens at high throughput making them particularly suitable for serological surveillance studies [38,39]. de Assis et al. [38] recently developed Corona Virus Antigen Microarrays (CoVAM) for the analysis of SARS-CoV-2 antibodies in COVID-19 convalescent blood. This assay was used in further studies to compare the antibody response induced by the vaccine and the natural infection of SARS-CoV-2 [7]. The steps of production and analysis of antigen microarrays are shown in Fig. 7.

v. Chemiluminescent ImmunoAssay (CLIA)

CLIA is a method for determining the concentration of a sample according to the intensity of the glow emitted by a chemical reaction. The basis of the CLIA method is similar to ELISA, but the CLIA substrate can produce light emissions in the presence of enzymes providing a more sensitive process than ELISA. Electro-chemiluminescence immunoassay

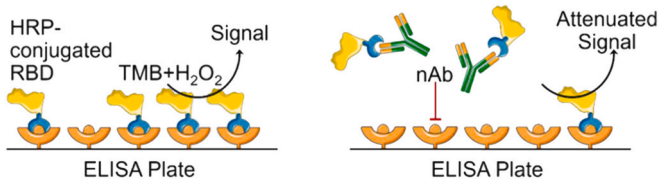


Fig. 4. ELISA assay with competitive binding principle between NAb and ACE2. Reprinted from Ref. [5] with Elsevier's free permission.

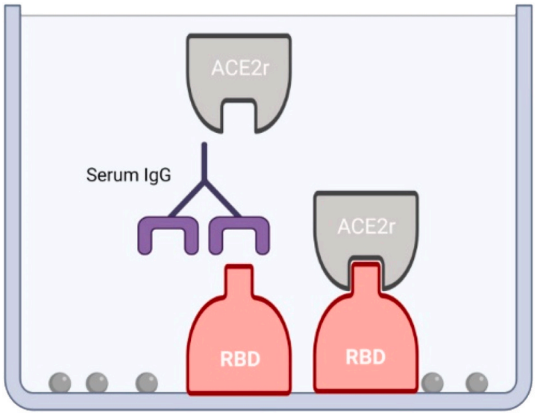


Fig. 5. ELISA test with reverse competitive binding principle. Reprinted from Ref. [9] with Creative Commons license.

(ECLIA) is another type of CLIA. ECLIA uses an electric current to oxidize the substrate. The CLIA and ECLIA methods have higher sensitivity than ELISA and ELFA and have a shorter analysis time [40,41]. CLIA has times ranging from 15 min to several hours. ELISA and CLIA have high throughput with a high analytical agreement rate [5,42].

Just like ELISA, CLIA also uses substrate and enzyme labels. The most commonly used substrates are luminol, isoluminol, and their derivatives, acridinium ester derivatives, peroxidase, and alkaline phosphatase (ALP). Isoluminol or acridinium esters produce a luminescent signal in the presence of hydrogen peroxide and enzymes. The most commonly used enzyme labels are horseradish peroxidase (HRP) and ALP [41].

Nanoparticles have good biological compatibility and signal amplification effect, nanotechnology has been widely used in bio-labeling. Nanotechnology has been widely used at CLIA to accelerate the rapid development of CLIA. Some nanoparticles can not only directly catalyze the chemiluminescent (CL) reaction as an enzyme imitator, but also load a large number of enzymes to achieve signal amplification [41].

vi. Electrochemical Biosensor

Biosensors are analytical devices that can convert biochemical reactions into detectable and measurable parameters [29,43]. Biosensors are the simplest, fastest, and high-throughput point-of-care technology for evaluating post-vaccination antibody levels [15,44]. The biosensor has two important components, namely a biological element and a transducer (Fig. 8). Biological elements/bioreceptors are responsible for recognizing analytes to generate biological signals. The transducer converts the biological signal into a detectable response [43,45].

Electrochemical biosensors can detect biomarkers with high accuracy, specificity, and sensitivity. The transduction element of the electrochemical biosensor is an electrochemical cell whose main component is a working electrode. The electrodes convert the recognition system into a measurable electroanalytical signal. Electrochemical biosensors based on antigen-antibody interactions are called immunosensors [43,47].

Screen-printed carbon electrode (SPCE) based electrochemical biosensors have sensitivity, the ability to work in complex matrices, and ease of use [48]. SPCE only requires a small number of samples, and is relatively inexpensive, portable, and easy to use because it is disposable [49]. In addition, SPCE is easily modified with nanoparticles to improve electroanalytical performance, stability, and sensitivity of the biosensor because SPCE has a narrow working electrode surface area [43,48,50]. Electrochemical biosensors with nanoparticles can increase the rate of electrochemical reactions due to an increase in the electrode surface area to volume ratio, thereby increasing the electrode surface area to the volume of the analyte liquid [51].

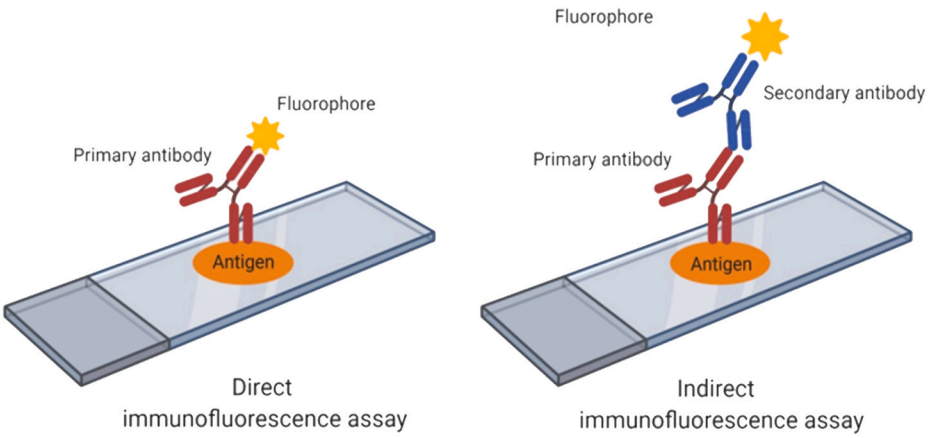


Fig. 6. Principles of direct and indirect IFA testing. Reprinted from Ref. [37] with CC-BY-NC license.

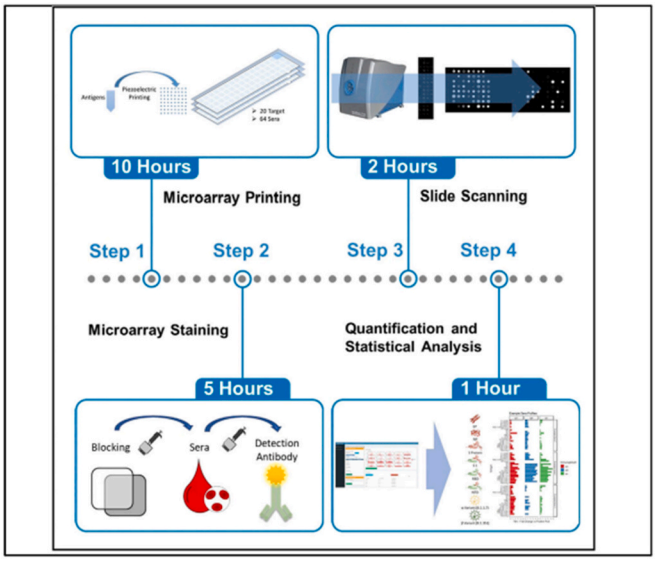


Fig. 7. The production and analysis steps of antigen microarrays include array printing, staining, measurement, and data analysis. Reprinted from Ref. [39] with CC-BY license.

Rahmati et al. [16] developed an SPCE-based label-free electrochemical immunosensor modified with nickel hydroxide nanoparticles ($Ni(OH)_2$ NP) for selective and quantitative analysis of SARS-CoV-2 virus antibodies. SPCE was surface activated with H_2SO_4 and electrodeposited using $Ni(OH)_2$ NP. Next, spike protein was added to bind covalently to the amine group on the protein to form $Ni(OH)_2$ NP-NH_2. Bovine Serum Albumin (BSA) was added to block non-specific binding. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) were used to analyze IgM/IgG antibodies using the $[Fe(CN)6]^{4-/3-}$ redox system (Fig. 9). This biosensor produces a low detection limit of 0.3 fg/ml and tests in 20 min.

Huang et al. [15] developed a nanoplasmonic immunosorbent assay (NanoPISA) biosensor for rapid one-step quantification of SARS-CoV-2 neutralizing antibody (NAb) in postvaccination individual serum samples. The combination of the nanoplasmonic nanocup sensor and NHGNP (nanoporous hollow gold nanoparticles) provides a stronger SPR (surface plasmon resonance) effect and higher sensitivity. This NanoPISA has a detection limit of 0.2 pM within 15 min and gives comparable results to the PRNT and ELISA assays. This makes it possible that NanoPISA can be used to evaluate vaccine effectiveness on a large scale.

The NanoPISA detection scheme is shown in Fig. 10. Anti-human IgG solution was coated on the surface of the biosensor in a 96-well plate modified with gold nanosheets. The sample serum and NHGNP-labeled RBD S were added sequentially resulting in the binding of SARS-CoV-2 NAb to serum positive with labeled RBD S. Then captured by anti-human IgG on the surface of the sensor to form a sandwich conjugate (antibody-protein-antibody). This conjugate produces an SPR effect via the plasmonic biosensor chip and changes the OD at a certain wavelength proportional to the SARS-CoV-2 NAb concentration in serum.

Several serological test methods have been reported and performed on human samples. Types, detection methods, vaccine types, and testing sites are summarized in Table 2.

1.2. *Post-vaccination antibody level response*

Antibody responses due to infection and vaccines are different and have become the focus of recent research. Several studies have also compared the response of antibody levels between two groups of vaccinated patients based on a history of previous SARS-CoV-2 infection. Factors of age, gender, and the background of the disease have been studied. Next, we will discuss the post-vaccination antibody response.

Assis et al. [7] compared individual antibody levels after mRNA vaccination with natural infection using the IFA serological method. The results obtained that mRNA vaccination induced significantly higher antibody levels against RBD than natural infection. Considering that the BNT162b2 vaccine provides mRNA encoding for the spike protein, the expected response is the production of antibodies against the spike protein [52]. Neutralizing antibody levels were found to be significantly higher in response to the vaccine than in natural infection. Therefore, mRNA vaccines are very effective in increasing antibody levels against the SARS-CoV-2 antigen.

Determination of antibody levels was carried out using the multiplex antigen microarray method containing the SARS-CoV-2, SARS, MERS, CoV, and Influenza antigens to determine the effectiveness of the mRNA vaccine against viral variants. The results suggest that the mRNA vaccine induces a cross-reactive response to SARS and SARS RBD whereas natural infection does not. This provides evidence that mRNA vaccines may be effective against emerging viral variants [7].

Two individual terms need to be understood in serological testing, namely individuals with a previous infection are called seropositive and without previous infection are called seronegative. Saadat et al. [10] studied antibody response to a single dose of Pfizer-BioNTech or Moderna vaccine in 59 seropositive and seronegative healthcare workers using the ELISA serological method. Antibodies to the nucleocapsid are used to differentiate individuals in the vaccinated population to classify seropositive and seronegative individuals. This is because the nucleocapsid is not a component of vaccine mRNA, so there is no increase in

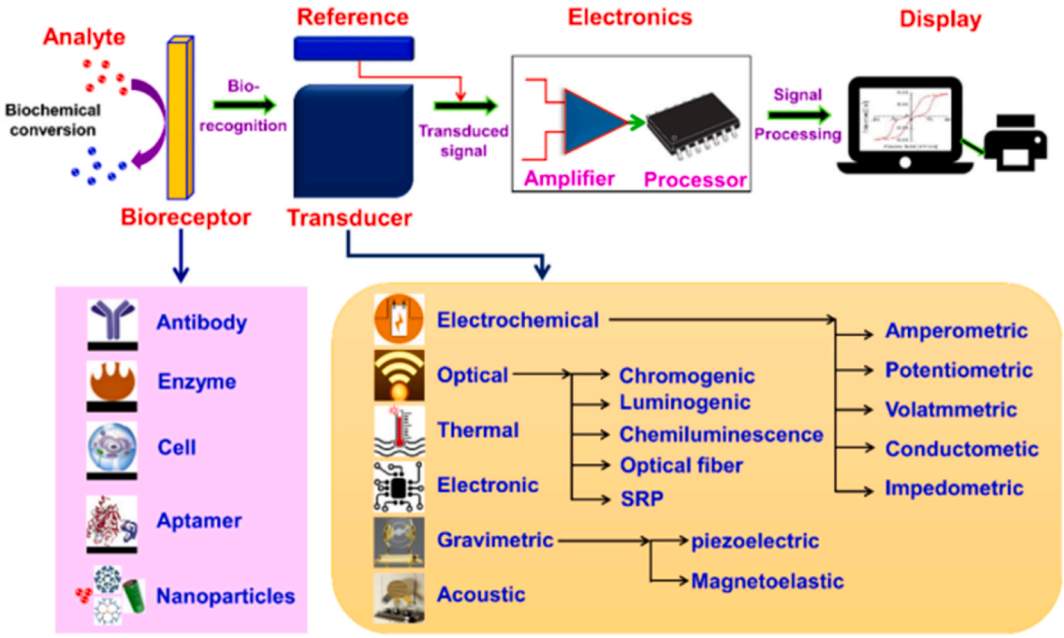


Fig. 8. Schematic of the biosensor. Various types of bioreceptors and transducers can be used in biosensors. Reprinted from Ref. [46] with CC-BY license.

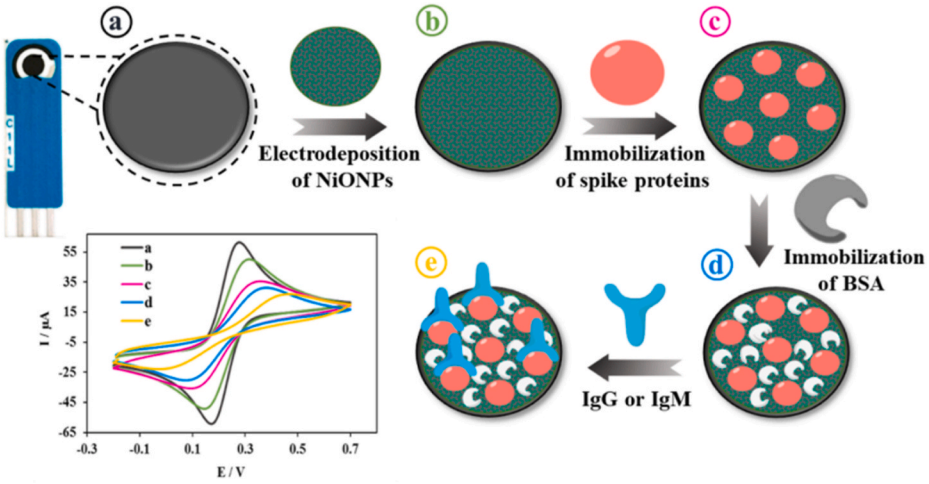


Fig. 9. Schematic of electrochemical immunosensor on SPCE for detecting SARS-CoV-2 antibody. Reprinted from Ref. [16] with Elsevier's free permission.

antibody to protein N induced by the vaccine. Thus, antibody to spike was increased and antibody to nucleocapsid was maintained in seropositive individuals [7]. The results showed that seropositive individuals had significantly higher levels of anti-S IgG antibodies than seronegative individuals (Fig. 11).

Study Gobbi et al. [53] evaluated the immune response of 15 health care workers (6 seropositive individuals and 9 seronegative individuals) after Pfizer vaccination using the CMIA serological method. It can be seen in Fig. 7 that individuals after SARS-CoV-2 infection experienced a progressive decrease in RBD IgG titers and neutralizing antibodies at 2–7 months. Results showed that seropositive antibody titers were significantly higher than seronegative ones at 7 and 21 days after the first dose (Fig. 12).

Ebinger et al. [52] studied antibody responses to the first and second doses of the BNT162b2 mRNA vaccine (Pfizer–BioNTech) in a large and diverse cohort of healthcare workers (n = 1090). The anti-RBD S IgG threshold used was 4160 AU/ml based on a 0.95 probability of obtaining a PRNT ID50 at a dilution of 1:250. The results showed that seropositive individuals had significantly higher antibody levels than seronegative individuals. There was no difference in anti-RBD S IgG levels between seropositive individuals after receiving the first vaccine dose and seronegative individuals after receiving the second vaccine dose ($P = 0.92$). IgG anti-RBD S in response to infection was significantly lower than vaccination at the first dose ($P < 0.001$) (Fig. 13).

Claro et al. [6] study of the Sputnik V vaccine gave similar results in that seropositive individuals had a strong immune response to the first vaccination and had little benefit from additional antibodies after the second vaccination. The first vaccination in seropositive individuals acts as a booster because there is a very high increase in titers after one week of the first vaccination [53]. 58% of seronegative individuals gave an anti-RBD IgG response after the first dose of vaccine and up to 100% of seronegative individuals had an anti-RBD IgG response. It is therefore important for seronegative individuals to receive 2 doses of the vaccine. Based on this, a new strategy for vaccines emerged where: a) a single dose of vaccine was administered to patients with a history of previous COVID-19 infection, thereby providing an opportunity to save on doses; b) patients with a history of previous COVID-19 infection may be placed on a lower vaccination priority list; c) extension of time from COVID-19

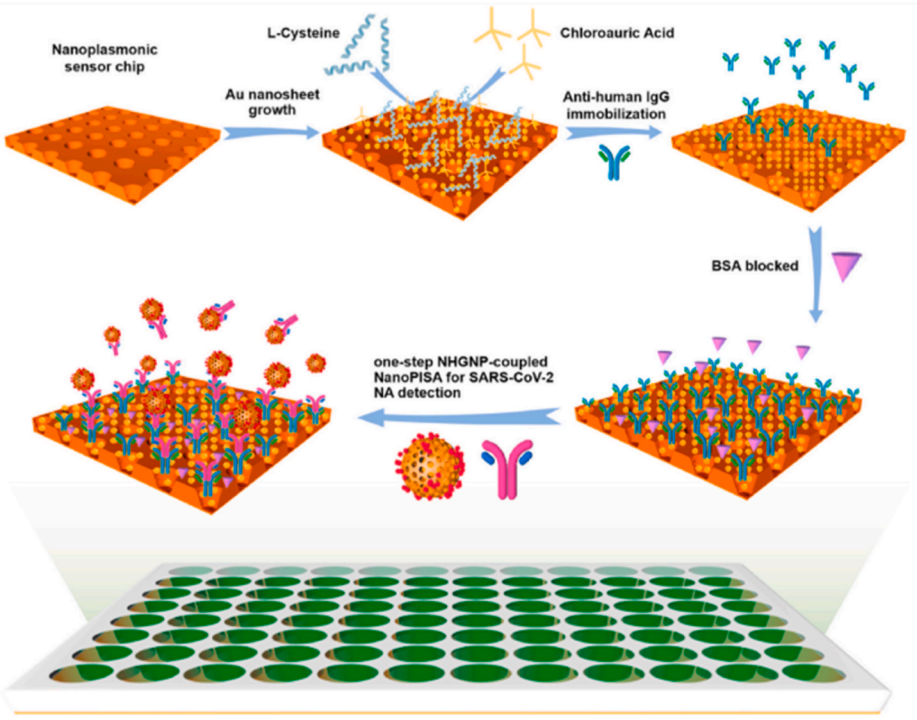


Fig. 10. Schematic of rapid one-step quantification of SARS-CoV-2 neutralizing antibody on a nanoparticle-coupled biosensor platform. Reprinted from Ref. [15] with Elsevier's free permission.

Table 2
Several methods of postvaccine SARS-Cov-2 serological tests have been reported.

Inspection Method	Limit of Detection	Vaccine	Country	Reference
ELISA	–	Pfizer and Moderna	USA	[9]
	–	Sputnik V	Venezuela	[6]
	–	Pfizer and Moderna	USA	[10]
	–	Pfizer and Moderna	USA	[32]
	0.54 BAU/mL	Pfizer and Moderna	USA	[33]
CMIA	4.3 AU/mL	Pfizer	USA	[20]
	4.3 AU/mL	Pfizer	USA	[52]
	6.9 AU/mL	Pfizer	Italy	[53]
IFA	–	Moderna	French	[54]
	–	Pfizer and Moderna	USA	[7]
CLIA	–	Pfizer	Israel	[55]
	0.717 AU/mL	Pfizer	Germany	[13]
	–	Pfizer	Israel	[56]
	–	Pfizer	Italy	[57]
ECLIA	0.35 U/mL	Pfizer and Moderna	USA	[58]
	0.35 U/mL	Pfizer and Moderna	Belgium	[59]
ELFA	–	CoronaVac	Turkey	[60]
Biosensor Modification	Limit of Detection	Testing time		Reference
SPCE-Ni$(OH)_2$	0.3 fg/ml	20 min		[16]
NanoPISA-Au	0.2 pM	15 min		[15]

infection to vaccination beyond the currently recommended 3 months [10].

But different results were shown by the research of Binay et al. [60] where the levels of IgG SARS-CoV-2 seropositive and seronegative gave no significant difference ($P > 0.05$) against the Sinovac vaccine, this

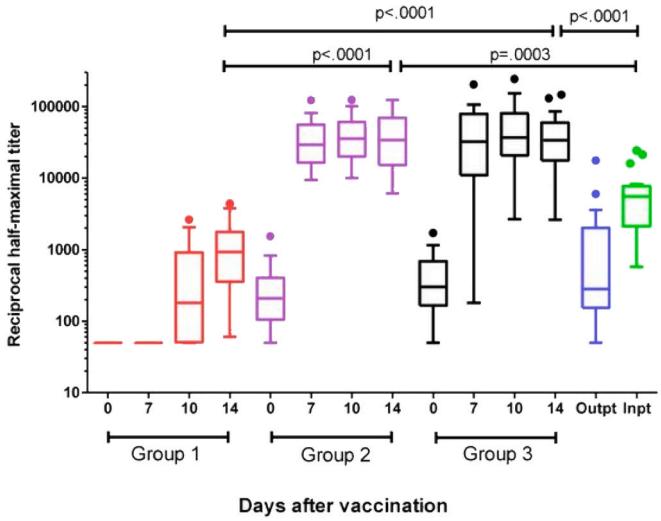
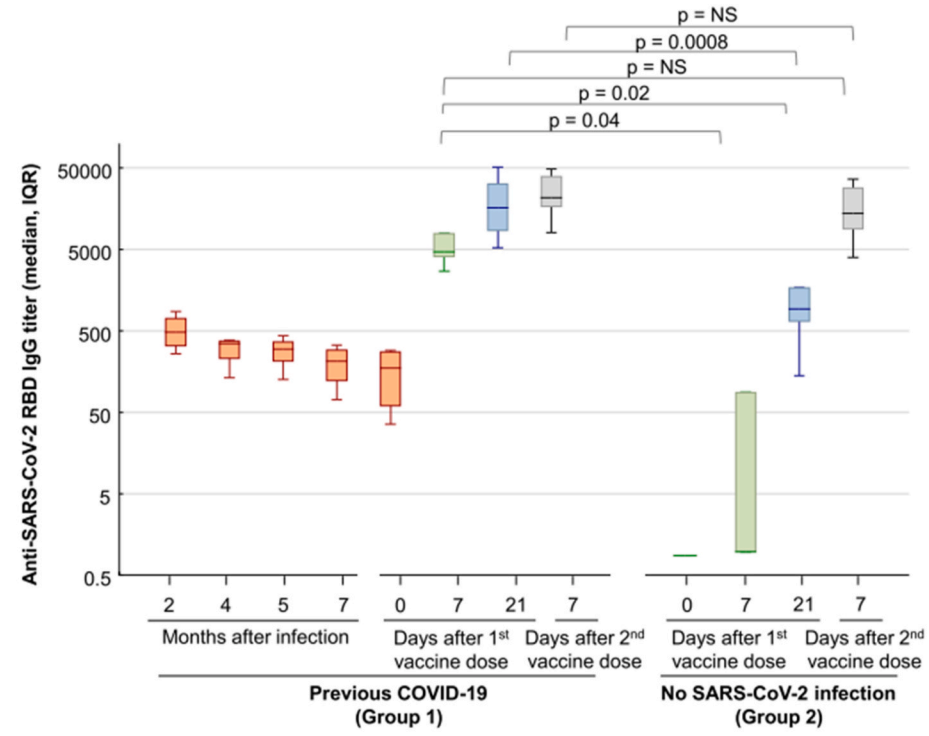


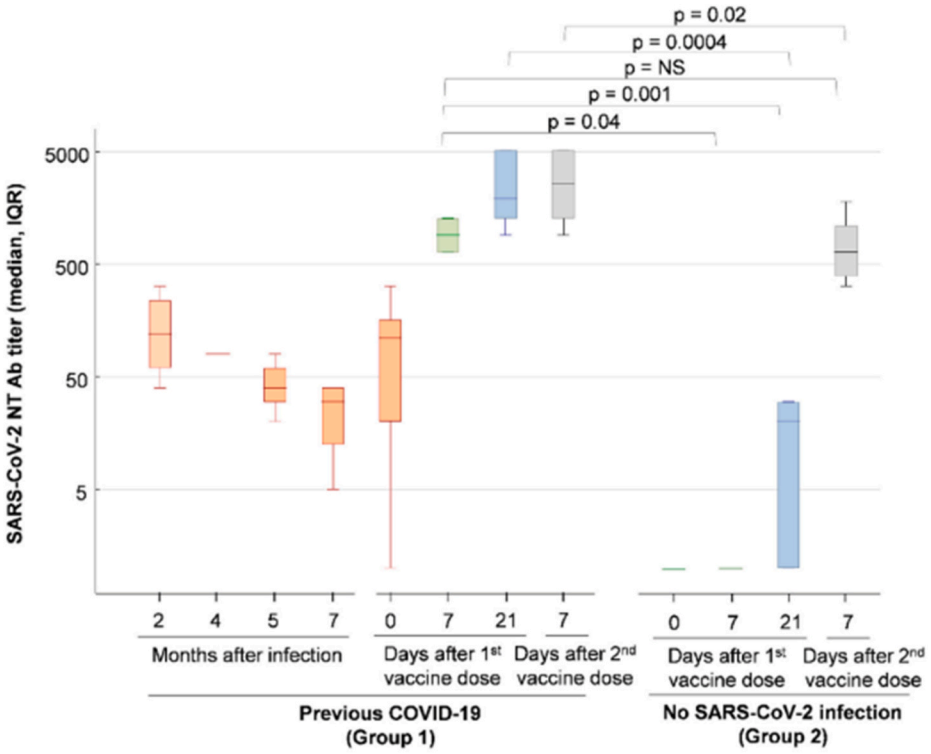
Fig. 11. Boxplot of postvaccination single postvaccination IgG anti-S SARS-CoV-2 antibody response. HCW (health care workers) = health workers. Group 1: HCW negative for IgG SARS-CoV-2. Group 2 = asymptomatic SARS-CoV-2 IgG positive HCW. Group 3 = HCW positive for IgG SARS-CoV-2 symptomatic. Reprinted from Ref. [10] with CC-BY–NC–ND 4.0 International license.

could be due to the selection of seropositive individuals infected in the 2–8 months before vaccination experiencing a progressive decrease in antibody. Some variation in antibody response is related to the heterogeneity of previously infected individuals (time and disease severity) and limited sample size [52].

Post-vaccine antibody titers were correlated with age. There are two categories of groups in receiving vaccines, namely the old age group (age >50 years) and the young age group. The old-age antibody titer showed a significantly lower value than the younger age group in the first dose of the vaccine. After the second dose of the vaccine gives a



a)



b)

Fig. 12. Response after natural infection and after vaccination with Pfizer COVID-19 vaccine a) IgG anti-RBD S b) NAb SARS-CoV-2. Reprinted from Ref. [53] with CC-BY license.

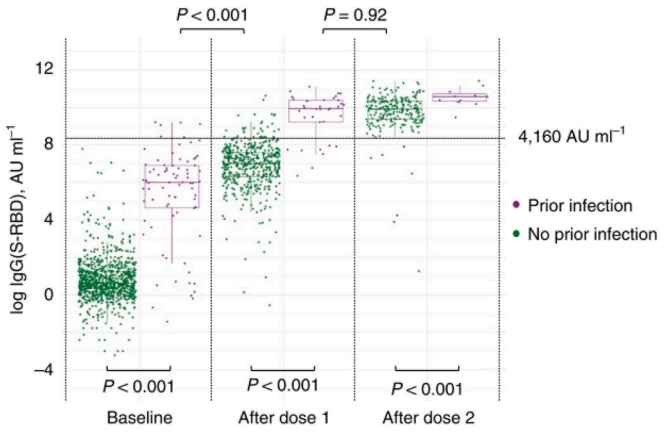


Fig. 13. Anti-RBD S IgG antibody response to SARS-CoV-2 mRNA vaccination in individuals with and without previous infection. Reprinted from Ref. [52] with Creative Commons 4.0 International license.

lower value by 30% compared to other age groups [6,60]. Older individuals usually have a less favorable response to vaccines due to aging immunity [61].

There were no statistically significant differences between male and female antibodies or individuals with diseases (hypertension, diabetes, and asthma) in the seronegative group [6]. Gender did not give a significant difference in the level of IgG SARS-CoV-2 ($p > 0.05$) [60].

Different results occurred in lymphoma patients where lymphoma patients were found to have a lower SARS-CoV-2 IgG response than healthy controls but not significantly. Individuals with different types of lymphoma had a nonsignificantly different antibody mean. However, all healthy individuals induce antibodies to the vaccine whereas most lymphoma patients do not (30/67). This is associated with commonly used lymphoma therapies that may affect the performance of COVID-19 vaccines. Lymphoma patients who were treatment-naive or had not received therapy for at least 2 years responded to vaccination like the control group. However, patients undergoing active therapy for lymphoma may not respond to vaccination [32].

Several serological test studies have also examined post-vaccine symptoms. Seropositive individuals were found to experience systemic symptoms after the first dose of vaccine more frequently than seronegative individuals. The most common symptoms experienced by seropositive individuals after the first dose are fever, chills, and local area tenderness (swelling, tenderness, and erythema). However, there was no significant difference in symptoms after the second vaccine between seropositive and seronegative individuals. This is because both seropositive and seronegative individuals have symptoms that appear more frequently after the second dose of the vaccine. The most common symptoms experienced by seronegative individuals are headaches and dizziness. This is associated with seronegative individuals having higher reactivity after the second dose of the vaccine [11,52,53].

2. Conclusion

Several review articles have discussed several detection methods. For example, Ilkhani et al. [62] have discussed new approaches for rapid detection of COVID-19 during the pandemic. This review provides different information from the previous review in that it discusses several serologic test methods and antibody titer responses that have been described after the COVID-19 vaccine. Serological testing is a technique for measuring a person's antibody levels to determine the human body's immune response to vaccines. The serological test methods that have been discussed include PRNT (gold standard), LFIA, ELISA, IFA, CLIA, ECLIA, and electrochemical biosensors. Among the various methods, electrochemical biosensors provide the highest chance of quantitatively determining antibodies due to their good detection limits. In addition, the advantages of electrochemical biosensors are mainly fast, sensitive, accurate, portable, and easy to use and are more profitable to be developed at this time [29,44,63,64]. Differences in antibody titer responses to vaccines are caused by several factors such as disease history, age, and gender. The antibody titer response to the vaccine differs depending on whether the individual has been previously infected or not. Seropositive individuals have higher antibody levels than seronegative individuals. Vaccination also induces much higher levels of RBD antibodies than natural infection. Age and gender differences do not provide significant results for vaccination. This review can provide an understanding of the application of the body's immune response to vaccines to get some new global COVID-19 vaccination strategies in 2023 and beyond.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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