Laboratory examination to measure antibodies formed after vaccination of COVID-19

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Abstract Coronavirus Disease (COVID-19) as the reemerging disease which first discovered on December 2019 in Wuhan, China. World Health Organization (WHO) has designated COVID-19 as a global pandemic on March 11th, 2020. Covid-19 is related with the climate change which influences the environment of life cycle of SARS-CoV2 vector. With there are no drug of choice for Covid-19 until this time, giving vaccination is one of the strategy of prevention to break the transmission and reduce the number of cases. Vaccination can trigger immunity by forming antibodies and protect the individual from antigen which came in to host and can be a marker for future vaccine for disease relates unpredicted climate change. This paper reviews several laboratory methods that can be used to detect antibodies such as Lateral Flow Immunoassay (LFIA), Enzyme-linked Immunosorbent Assay (ELISA) and Electro-Chemiluminescence Immunoassay (ECLIA) and see their ability to protect individuals from COVID-19 after vaccination using neutralization test such as Plaque Reduction Neutralization Test (PNRT) or Surrogate Reduction Neutralization Test (sRNT). The benefit of this reviews is to understand the optimal methods to measure and detect antibody and to improve vaccine development strategy for the disease relate with unpredicted climate change in the future based on antibodies seroconversion and seroprotection.

1. Introduction

Coronavirus Disease (COVID-19) as the reemerging disease which first discovered on December 2019 in Wuhan, China. World Health Organization (WHO) has designated COVID-19 as a global pandemic on March 11, 2020. SARS-CoV2 as the cause has disturbed everything from the health, social to economic systems which has an impact on increasing mortality, morbidity, unemployment, and poverty [1]. So far, the Covid cases reported through the worldmeter have reached more than 150 million with cases of death reaching 3 million. Meanwhile, cases of Covid-19 infection in Indonesia have reached 1.7 million and deaths of up to 40 thousand cases.

Covid-19 is related with the climate change which influences the environment of life cycle of SARS-CoV2 vector. Climate change has significant impacts on humans, pathogens and the environment [2]. Climate change has increased or changed an ecosystem resulting in changes from living things to survival, including pathogens and vectors [3]. Thus it can be predicted that there will be changes in the transmission of the spread of the disease as mentioned previously [4], which has an impact on changes in management patterns that require time to get standard treatment.
With there are no drug of choice for Covid-19 until this time, giving vaccination is one of the strategy of prevention to break the transmission and reduce the number of cases. Vaccination can trigger immunity by forming antibodies, and protect the individual from antigen which came in to host and can be a marker for future vaccine for disease relates unpredicted climate change. The presence of antibodies is very important to know to ensure that prevention is successful. Learning from Covid-19, many vaccines have been developed but their efficacy still varies [5]. To assess its success, the antibodies that are expected to be formed must be measured so that the hope of preventing infection from new emerging and emerging diseases can be achieved.

This paper reviews several laboratory methods that can be used to detect antibodies such as Lateral Flow Immunoassay (LFIA), Enzyme-linked Immunosorbent Assay (ELISA) and Electro-Chemiluminescence Immunoassay (ECLIA) and see their ability to protect individuals from COVID-19 after vaccination using neutralization test such as Plaque Reduction Neutralization Test (PNRT) or Surrogate Reduction Neutralization Test (sRNT). This writing paper is a literature review from books and articles research results, systematic reviews or literature reviews, guidelines and others. The topics assessed were literature related to climate change, reemerging diseases and new emerging.

2. Re-emerging and new emerging disease in era climate change
Climate change has had an impact on changes for humans, agents and the environment and this has triggered the development of re-emerging and emerging diseases. Climate change affects climate variables such as temperature, wind, humidity, and even sun exposure which results in ozone damage, biodiversity disturbances, changes in ecosystems, hydrological systems, limited food production and this affects living things including vectors and disease pathogens such as affect the reproductive system, the ability to survive, transmission, the level of virulence which ultimately has an impact on the level of human health [2, 3]. Other pathogenic changes that can occur are the triggered increase in pathogen cytotoxicity, increased levels of host susceptibility, rapid disease transmission, changes in host pathogens from animals to humans, the evolution of disease types [2].

The temperature changes that occur are said to have an impact on the life cycle of pathogens, from the ability to survive to reproduction [3,8]. Increasing temperatures due to global warming will certainly make the world temperature rise and have an impact on the adaptation efforts of pathogens and vectors to survive. Other factors such as precipitation events will increase the problem of water-borne disease. The same thing happens to the problem of responsive weather humidity, one of which can trigger an influenza pandemic [3].

SARS-CoV2, which is currently a pandemic, is also suspected of having occurred because of a change in the causing vector. Climate change is said to change bushes into savanna and tropical forests which increase the number of bats, where this also triggers changes that occur in the body of bats, including bats carrying CoV, where cross species can occur thereby facilitating the movement to humans [8]. Other diseases such as Middle East respiratory syndrome corona-virus (MERS-CoV), human coronaviruses, Avian influenza, Ebola, dengue fever, malaria, ticks and others are also diseases whose presence can have a global impact and are influenced by climate change [9].

3. Vaccine in re-emerging and new emerging disease
Immunization is one of the strategies to overcome Re-emerging and New emerging Diseases. To get the ideal vaccine against Covid-19, SARS or pandemic flu, anthrax, Ebola, malaria, HIV and other emerging-related diseases is a challenge that must be faced by various sectors. Learning from the current Covid-19 era, it turns out that the opportunity to get the ideal vaccine is very open, it can be seen from the development of vaccine development techniques that utilize various methods of making vaccines.

Various types of vaccines such as inactivated, live attenuated, polysaccharide, subunit, conjugate, nucleic acid, re-combinate, adenovirus based and toxoid vaccines can be an option for reemerging and emerging diseases [7]. However, to get a vaccine with high efficacy it takes a long time and must meet the requirements in accordance with the Drug Control Agency. In addition, developing a vaccine with high efficacy is not an easy matter. This can be seen from the findings of Covid-19 vaccines such as
vaccines made by Astra Zenica, Johnson & Johnson, Moderna and Pfizer with efficacy ranging from 67% - 95%, and with minimal adverse events [5]. In addition there is Russia's Sputnik V Vaccine which is said to have reached efficacy [9], and the Chinese vaccine made by Sinovac shows efficacy results ranging from 55.65% to 83.5% based on reports from Brazil, Turkey and Indonesia, while sinopharm is reported to be 76.34% based on interim results from the Sinopharm subsidiary China National Biotec Group (CNBG) [10].

4. Response antibodies post vaccination
When vaccination is carried out, it is hoped that an immune system will be formed that remembers and recognizes the pathogen when it invades the host. The body's defense system that is familiar with the pathogen is expected to react immediately after contact with the pathogen. The immune response of the antibodies to the incoming pathogens is the main basis for vaccine development. In the process, antibodies will bind to pathogens and carry out their effector duties, as well as other immune systems to carry out their effector mechanisms with their respective pathways. This is what is expected from vaccines by inducing the effector mechanism of the immune system.

Antibodies that are formed in the body, both post-infection and post-vaccination, are tasked with preventing or reducing infection through mechanisms to prevent pathogen diffusion, neutralization of viral replication, bacterial opsonopagocytosis, and complement activation [11]. Furthermore, it is stated that the injected vaccine antigen will translocate in the B cell zone, and will bind to B cells which will then interact with T cells which will trigger the proliferation of B cells to become antibody-secreting plasma cells or memory.

Antibody secreting plasma cells are responsible for preventing infection in the host body, which occurs when an antigen enters. However, if it does not enter the body, these antibodies can last for some time depending on the vaccine received, it is said that some can last 6-12 months [11]. The long duration of whether or not these antibodies depend on the type of vaccine, adjuvant, generality, schedule of administration, while only the live vaccine type or viral particles have a longer duration [11].

Memory antibodies act not protection but will rapidly proliferate to become antibody secreting plasma when interacting with antigens. These memory cells travel around the spleen and nodes through the bloodstream where after vaccination their presence will go to the lymphoid organs. The rate of change in B memory cells due to their response to antigens in the absence of a CD4 role is a marker of secondary response to the presence of pathogens. In addition, the antibodies produced have a higher affinity than antibodies due to primary infection [11]. The presence of memory B cells is important in the immunization program, so a strategy is needed to defend them in the host. Secondary / booster doses or adjuvant use play a role in inducing memory B cells, including the time interval for booster administration is said to affect the affinity of memory B cells [11].

5. Antibody laboratory measurement
Antibodies can be formed after previous COVID-19 infection especially in mild infections or post vaccination. An antibody response is associated with more severe clinical disease, while a T-cell response is associated with less severe disease [12]. There can be detected at day 10 - 15 or more after the onset of symptoms of infections. Because of that, we can not test for antibodies formed when COVID-19 infection occurs immediately; the sensitivity is too low in the first week of symptoms, this information plays an important role in the diagnosis of COVID-19. However, the duration of the increase in antibodies is not known and after 35 days after starting the symptoms are usually less noticeable [13].

Antibody Laboratory measurement develop to detect seroconversion of immunoglobulin G (IgG) and immunoglobulin M (IgM) which occurs sequentially or simultaneously after infection or vaccination. The seroconversions of immunoglobulin show how much antibodies titter found at or body, the higher of antibody it can be describe as a capacity of antibody to fight the antigen/pathogen. But not only is the titter, an important rule of that whether the antibodies that are formed will provide adequate protection from future infections [14].
The most commonly used serologic methods are Enzyme-linked Immunosorbent Assay (ELISA), Electro-Chemiluminescence Immunoassay (ECLIA) and Lateral Flow Immunoassay (LFIA). This method can detect IgM, IgA, IgG or total antibodies. The good time to detect antibodies as explain before is performed 10 – 15 days or 2 weeks after symptom onset. This measure is more accurate than those performed earlier when symptoms appeared expectedly.

The assay varies on the specific antibody detected, including antibodies to RBD, nucleocapsid protein (N), spike protein (S), or nucleocapsid and spike (NS) protein [15]. The LFIA method is a rapid immunochromatography-based method that uses conjugated gold colloid SARS-CoV-2 antigen. The LFIA examination usually requires only a few drops of blood placed on the test strip. The sample migrates towards the fixed band of antigen-bound SARS-CoV-2. If the sample contains SARS-CoV-2 specific antibodies, these antibodies will bind to the antigen and produce a visible band [15]. The LFIA method is the method used in most rapid tests. Examination with LFIA has several advantages, including an established method, ease of production, stable (12-24 months without refrigeration), easy to use, and relatively inexpensive cost, and can use capillary blood. However, this method is generally only qualitative in nature. In addition, there are still very few reports on the performance of using rapid test diagnostics and most studies only use a small sample [16].

ELISA method is mostly used for antigen, hapten or antibody detection. Its working principle is based on a specific reaction between antigen and antibody which has high sensitivity and specificity by using enzymes as indicators. The enzyme will react if the antigen reacts with the antibody. This reaction requires specific antibodies that bind to antigens [16]. After the sample is added, the antigen-specific antibody binds to the antigen. After washing, the conjugate that binds to the antigen-antibody complex is added. A substrate is added, which will react by conjugation, resulting in a color change. The magnitude of the color change is a quantitative measure of the number of antibodies present in the sample. ELISA easily adapts automation for high yields [15].

The CLIA method is a method for determining the concentration of a sample according to the intensity of the luminescence emitted by a chemical reaction. In general, a chemiluminescence reaction produces one of the reaction products in an electronically excited state which produces light when it falls to the ground state. The light emission process in chemiluminescence is the same as in photoluminescence, except in the excitation process. In fluorescence and fluorescence, the excited state is electronically generated by the absorption of UV visible light which returns to the ground state (S0) from the lowest singlet excited state (S1) or from the triplet excited state (T1) [16].

The results of the analysis show that the CLIA method offers a more accurate diagnosis than ELISA or LFIA [17]. The CLIA method was observed to be superior to ELISA and LFIA in detecting total antibodies and was suitable for all stages of the disease. The diagnostic efficiency of CLIA was found to be higher than that of LFIA and ELISA. The CLIA method shows excellent sensitivity and specificity in detecting IgG and IgM antibodies. The overall diagnostic efficacy of CLIA is higher than ELISA, LFIA and FIA [18].

The sensitivity of the LFIA method is lower than that of the ELISA and CLIA methods. The type of immunoglobulin detected such as IgM, IgG, or both did not correlate with diagnostic accuracy. Specificity was lower in individuals with suspected COVID-19. Other viral infections can cause false positives on the LFIA method. Serological methods have drawbacks, especially when used as a place of care [19]. A study shows that the sensitivity of IgG and IgM with ELISA is lower than that using CLIA [20].

The neutralization test for antibodies is carried out to determine herd immunity and humoral protective immunity in vaccine survivors and recipients. The common standard for conducting antibody neutralization tests at present is the Plaque Reduction Neutralization Test (PRNT). This method requires a BSL3 laboratory, requires many processes, must use highly competent personnel, has a low yield rate and takes a few days. The Surrogate Virus Neutralization Test (sVNT) examination method is free from the use of replicated or cultured viruses and uses Enzyme-Linked Immunosorbent Assays (ELISA), so it is possible to get multiple results, automatic processing and fast turnaround times [21].

The PRNT method is a serological test that utilizes the ability of specific antibodies to neutralize viruses and prevent viruses from causing plaque formation in single cell layers. Typically, this testing involves
mixing a constant amount of virus with a dilution of the serum specimen and followed by coating the mixture onto cells of the corresponding cell lines for each virus. The concentration of plaque-forming units can be determined from the amount of plaque that forms after a few days. A vital stain is added for plaque visualization and the amount of plaque in individual plates is divided by the number of native virions to calculate the percentage of neutralization. Plaque-forming units are measured by microscopic observation, fluorescent antibodies, or specific dyes that react with infected cells. Interpretation is usually based on 70% neutralization, which is the last serum dilution capable of inhibiting 70% of total plaque (virions).

The PRNT method is considered the "gold standard" for detecting and measuring antibodies that can neutralize viruses that cause many diseases. This method has a higher sensitivity and is more specific than other serological methods for the diagnosis of multiple viruses.

6. Conclusion
Serological tests provide information about previous COVID-19 infections or vaccinations. They are not suitable as stand-alone diagnostics for acute-phase infections. However, in cases of more prolonged existing symptoms and when molecular diagnostic results are unavailable or inconclusive, serological diagnostics could identify additional COVID-19 cases among suspected patients. Awareness of the limitations of serological and immunological tests in a particular setting is required, because of the large heterogeneity in test performance of different assays. Currently, it remains uncertain how serological and immunological parameters are precisely correlated with the extent of protective immunity.

The benefit of this reviews is to understand the optimal methods to measure and detect antibody and to improve vaccine development strategy for the disease relate with unpredicted climate change in the future based on antibodies seroconversion and seroprotection.

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