

Validity of Chemiluminescent Immunoassay Serology Test for Anti-Sars Cov-2 Antibodies IgM and IgG

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ABSTRACT

In December 2019, an outbreak of acute pneumonia occurred in Wuhan, China. The disease was transmitted between humans through droplets (coughing or sneezing) of infected patients, causing this outbreak to spread rapidly in various countries in the world, including Indonesia. On February 11, 2020, WHO announced the pneumonia was caused by Coronavirus Disease 2019 (COVID-19), which was caused by a new type of Coronavirus, the SARS-CoV-2. A rapid and accurate diagnosis is critical for the control of the COVID-19 outbreak. The widely used test is a serology-based test that detects the presence of SARS-CoV-2 IgM/IgG antibodies in the patient's body. One of the methods used for this test is Chemiluminescent Immunoassay (CLIA). This study aimed to determine the reliability of CLIA. The study was conducted from August to September 2020. The number of samples was 63 patients' serum. Polymerase chain reaction examination at Husada Utama Hospital, Surabaya, revealed that 21 patients were confirmed positive for COVID-19 with positive PCR results, and 42 patients were healthy with negative COVID-19 results. The results showed that IgM had a diagnostic sensitivity of 85.7%, diagnostic specificity of 92.8%, a positive predictive value of 85.7%, a negative predictive value of 92.8%, and accuracy of 90.4%. In comparison, IgG had a diagnostic sensitivity of 90.4%, diagnostic specificity of 90.4%, a positive predictive value of 82.6%, a negative predictive value of 90.5%, and accuracy of 90.4%. In conclusion, IgG has a higher sensitivity than IgM, while IgM had higher specificity, positive predictive value, and negative predictive value than IgG. However, the positive, negative predictive value and efficiency values were the same for IgM and IgG.

Keywords: Antibody, IgM, IgG, CLIA

INTRODUCTION

At the end of December 2020, an outbreak of acute pneumonia of unknown cause occurred in Wuhan, China.¹ Initially, this outbreak was thought to be transmitted from animals to humans (zoonosis). Transmission of this disease occurs between humans through droplets or direct contact with coughing or sneezing infected patients, causing a rapid and aggressive plague transmission.² On February 11, 2020, WHO named pneumonia Coronavirus Disease 2019 (COVID-19), which was caused by a new type of Coronavirus, the SARS-CoV-2.^{1,3} COVID-19, rapidly spread to various countries in the world, including Indonesia. On October 1, 2020, data stated that the number of confirmed COVID-19 cases in the world was 33,22,075 cases, with a death rate of 1,009,270 cases (CFR: 2.99%). The number of COVID-19 cases in Indonesia as of October 1, 2020, was 291,182 cases, with a death rate of 10,856 cases (CFR: 2.68%).¹

The incubation period for the SARS-CoV-2 virus is around 3-14 days (median five days). Symptoms of

COVID-19 such as dry cough, anosmia, fever, diarrhea, sore throat, fatigue, conjunctivitis, nausea, vomiting, shortness of breath, and sepsis appear after this period. There are no pathognomic symptoms of COVID-19 infection, so it is difficult to distinguish it from other respiratory viral infections.⁴ According to Ozdemi *et al.*, clinical manifestations of COVID-19 can develop into pneumonia, respiratory failure, and even death. About 80% of cases were classified as mild or moderate, 13.8% were seriously ill, and as many as 6.1% of the patients fell into critical condition. Worsening and death generally occur in older people with congenital diseases (50-75%).⁵

The conditions above show that a rapid and accurate diagnosis and control of the COVID-19 outbreak is crucial. The WHO recommended examination is a molecular examination using nucleic acid amplification or PCR or Real-Time Polymerase Chain Reaction (RT-PCR).³ The purpose of the RT-PCR study is to see if the virus is in a person's body and the sample is said to be positive (confirmation of the SARS-CoV-2 virus) if the RT-PCR

is positive for at least two genome targets (N, E, S, ORF1ab or RdRP).² However, the tests currently being carried out in the field are antibody-based tests, which are tests to detect the presence of IgM and IgG antibodies against the SARS-CoV-2 virus, one of which uses Chemiluminescent Immunoassay (CLIA).

Chemiluminescent immunoassay functions to quantitatively detect the presence of IgM and IgG anti-SARS-CoV-2 antibodies. It is widely known that immunoglobulin M (IgM) is the first line of defense during viral infection before the emergence of immunoglobulin G (IgG). IgM can be detected in the patient's blood about 3-6 days, while IgG can be detected 8-13 days after SARS-CoV virus infection. IgM antibodies tend to indicate a recent infection with the SARS-CoV-2 virus, whereas IgG shows past exposure. Therefore, detection of these antibodies is essential to provide information on the time course of the illness.⁶ IgM and IgG antibody-based examinations with the CLIA method are mostly carried out as a screening instrument for COVID-19. However, research on the reliability of this examination instrument has not been widely conducted.

METHODS

The number of samples in this study was 63 taken from the serum of Outpatients and Inpatients at Husada Utama Hospital Surabaya, consisting of 21 patients with confirmed COVID-19 and positive PCR results. All positive samples were tested more than five days after the onset of COVID-19 symptoms, and 42 healthy patients who were free of the SARS-CoV-2 virus confirmed with negative PCR results. The samples were examined for SARS-CoV-2 IgM and IgG antibodies using Mindray CL-Series SARS-CoV-2 CLIA IgM and IgG test to determine the levels of IgM and IgG antibodies. Calculations were carried out to assess the reliability of the examination, which included sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.

The examination followed the instructions of the Mindray CL-Series SARS-CoV-2 IgM/IgG with reagent kit lot number 2020060121 to detect IgM/IgG antibodies against SARS-CoV-2 quantitatively. Another protein that has a vital role in the antigenic site of the virus is the N protein, which is the nucleocapsid helix structural protein. N protein has a role in viral pathogenesis, replication, and packaging of RNA. Antibodies to protein N are frequently detected in COVID-19 patients. First, 10 µL of the sample solution treated with paramagnetic microparticle samples were coated with SARS-CoV-2

antigen, added to the reaction vessel, then incubated. After the incubation was complete, SARS-CoV-2 IgM/IgG bound to SARS-CoV-2 antigen-coated microparticles. The microparticles were captured magnetically, and unbound substances were removed by washing. Next, the diluent solution and ALP labeled anti-human IgM/IgG monoclonal antibody was added to the reaction vessel. After incubation, the IgM/IgG monoclonal antibody formed a sandwich structure with microparticles that captured the SARS-CoV-2 IgM/IgG antibody. The microparticles were captured magnetically, and unbound substances were removed by washing. Next, the substrate solution was added to the reaction vessel, which was catalyzed by IgM/IgG-ALP antibody. The conjugate formed the complex binding that was maintained on the microparticles. The chemiluminescent reaction product was measured as Relative Light Units (RLUs) by the photomultiplier on the instrument. The SARS-CoV-2 IgM/IgG antibody present in the sample was proportional to the RLUs generated during the reaction. The interpretation of this instrument was IgM was non-reactive if the level was < 1.0 COI, while IgG was non-reactive if the level was < 10.0 COI.

Approval of ethical eligibility was obtained from the Health Research Ethics Commission (KEPK) of Faculty of Medicine, Universitas Airlangga, with Number 273/EC/KEPK/FKUA/2020.

RESULTS AND DISCUSSIONS

The number of confirmed COVID-19 patients was 21. Table 1 shows 18 patients (85.7%) with reactive IgM with the minimum level of 1.11 COI, the maximum level 7.42 COI, and the mean reactive IgM was 3.69 COI, while non-reactive IgM was found in 3 patients (14.3%) with a minimum level of 0.45 COI, maximum level 0.55 COI, and the mean was 0.51 COI. The number of reactive IgG patients was 19 (90.5%) with a minimum level of 14.93 U/mL, a maximum level of 424.34 U/mL, and a mean level of 136.75 U/mL. There were two non-reactive IgG patients (9.5%) with a maximum of 1.55 U/mL, a minimum level of 0.17 U/mL, and a mean of 0.86 U/mL.

There were 42 healthy patients free of the SARS-CoV-2 virus. Table 2 shows that reactive IgM was found in 3 patients (7.1%) with a minimum level of reactive IgM of 1.19 COI, a maximum level of 4.42 COI, and a mean of 2.31 COI. Non-reactive IgM was found in 39 patients (92.9%) with a minimum level of 0.00 COI, a maximum level of 0.97 COI, and a mean of 0.28 COI. The number of reactive IgG patients was 4 (9.5%) with a minimum level of 50.52 U/mL, the

Table 1. IgM and IgG in 21 samples of COVID-19 cases with positive PCR results

Antibody	Total	Minimum Level	Maximum Level	Mean	Total Samples
Reactive IgM	18	1.11	7.42	3.69	21
Non-reactive IgM	3	0.45	0.55	0.51	
Reactive IgG	19	14.93	424,34	136,75	
Non-reactive IgG	2	0.17	1.55	0.86	

Table 2. IgM and IgG in 42 samples with negative PCR results

Antibody	Total	Minimum Level	Maximum Level	Mean	Total Samples
Reactive IgM	3	1.19	4.42	2.31	42
Non-reactive IgM	39	0.00	0.97	0.28	
Reactive IgG	4	50.52	202,68	110,71	
Non-reactive IgG	38	0.00	9.99	1.70	

Table 3. Validity of Sars-CoV-2 IgM and IgG antibodies

Antibody Types	Diagnostic Sensitivity	Diagnostic Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic Efficiency
IgM	85.7%	92.8%	85.7%	92.8%	90.4%
IgG	90.4%	90.4%	82.6%	90.5%	90.4%

maximum level was 202.68 U/mL, and the mean was 110.71 U/mL. Non-reactive IgG samples were found in 38 patients (90.5%) with a minimum level of 0.00 U/mL, a maximum level of 9.99 U/mL, and a mean non-reactive IgG level of 1.70 U/mL.

The calculation of IgM and IgG reliability of SARS-CoV-2 CLIA in Table 3 shows diagnostic sensitivity of 85.7%, diagnostic specificity of 92.8%, the positive predictive value of 85.7%, and negative predictive value of negative predictive value 92.8%, and accuracy of 90.4%. On the other hand, IgG has a diagnostic sensitivity of 90.4%, diagnostic specificity of 90.4%, a positive predictive value of 82.6%, a negative predictive value of 90.5%, and an accuracy of 90.4%.

This study aimed to determine the reliability of the CLIA test for anti-SARS-CoV-2 IgM and IgG. The results of this study indicated that false-positive IgM antibodies were found in three patients with a mean antibody level of 1.19 U/mL. IgG false-positive antibody was found in four patients with a mean antibody level of 202.68. In comparison, a false-negative IgM antibody was found in 2 patients with a mean IgG antibody level of 0.17 U/mL. False-negative IgG antibodies were found in three patients with mean IgM antibody levels of 0.55 U/mL.

False positives for IgM antibodies may be due to past infection. Likewise, false positives for IgG may also be due to past infections. It has been recognized that in patients who have recovered from COVID-19, IgG antibodies can remain in the body for several

months. False negatives in COVID-19 patients maybe because at the time of examination, the patient's IgM and IgG antibodies were still in the early days of being infected with the SARS-CoV-2 virus, and the virus was still in the incubation period causing the PCR examination results to be positive, but IgM and IgG were non-reactive because the body has not produced antibodies against SARS-CoV-2 virus. According to Hoffman *et al.*, seroconversion in COVID-19 patients occur between 7-12 days after symptoms appear. IgM is usually the first antibody to be produced, while IgG is produced later. However, studies on SARS-CoV also show that IgM and IgG often develop around the same time.⁷ According to Zhang *et al.*, immunity is usually stimulated by an increase in IgM levels after infection. IgG usually appears 1-2 weeks after and will remain in the body for a long time. The antibodies are specific to the SARS-CoV-2 virus. The rate at which anti-SARS-CoV-2 antibodies increase is different for each individual. In patients with mild clinical symptoms, particular antibodies appear early, usually on day 7. IgM is lower, and IgG continues to increase. In patients with severe symptoms, SARS-CoV-2 antibody reactivity occurs on day 12, and IgM continues to increase.⁸

Barbosa *et al.* stated that 2 confirmed COVID-19 patients whose IgM and IgG test results were negative. This result could be due to seroconversion from COVID-19 patients. However, the performance problem related to the exact time to determine a person's immune response after being infected with

the SARS-CoV-2 virus is still unknown.⁹

The calculation of the validity of the CLIA test on antibodies showed that IgM had a sensitivity of 85.7%, which was lower than IgG, with a sensitivity of 90.4%. However, IgM had a higher specificity than IgG, which was 92.% vs. 90.4%. The positive predictive value for IgM was 85.2%, and its negative predictive value was 92.8%. This finding showed that the positive and negative predictive value of IgM was higher than IgG, which only had a positive predictive value of 82.6% and a negative predictive value of 90.5%. In comparison, the accuracy of IgM and IgG tests had the same value of 90.4%. The results of this study were almost the same as those of Hoffman *et al.*, who stated that the sensitivity and specificity of IgG evaluated by Enzyme-Linked Immunoassay (ELISA) had a sensitivity of 97.5%. According to Li *et al.*, this examination is not appropriate for a diagnostic instrument. However, this examination has a role in detecting asymptomatic infections. For screening and surveillance purposes in the epidemiology of COVID-19.¹⁰ A sufficiently high specificity value indicates that this test helps detect past infections and is possibly crucial for social recovery.⁷ Researchers could not determine the seroconversion of anti-SARS-CoV-2 IgM and IgG antibodies, so the best seroconversion and time to take samples of SARS-CoV-2 IgM and IgG antibodies could not be determined. In the negative PCR samples, other diseases apart from COVID-19 could not be determined.

According to Barbosa *et al.*, the increase in SARS-CoV-2 antibodies is different for each individual.⁹ In patients with mild clinical symptoms, specific antibodies appear earlier, usually on day seven where IgM is lower, and IgG continues to increase; in patients with mild clinical symptoms and severe clinical symptoms of SARS-CoV-2 antibody seroconversion appear longer, usually on day 12 and IgM continues to increase.¹¹

According to Hoffman *et al.*, seroconversion in COVID-19 patients occurs between 7-12 days after the onset of symptoms. IgM is usually produced first and IgG later. The IgG lasts a long time in the body. Hsueh added that IgG seroconversion occurred on average ten days after the onset of clinical symptoms in COVID-19 patients, and the peak of the seroconversion was at 15 days. According to Doha *et al.*, seroconversion occurred sequentially for IgM and then IgG with a median time of 11 and 14 days, respectively, so if the sample were taken before that, it is possible that antibodies have not been formed, causing the tests to be false negative. According to research by Long *et al.*, seroconversion in 26 patients

who were initially seronegative during the observation period, there were three types of seroconversion, namely synchronous seroconversion of IgG and IgM, IgM seroconversion earlier than IgG and IgM seroconversion slower than IgG.¹² According to Li *et al.*, IgM could be detected in the blood of a person infected with the SARS-CoV2 virus for 3-6 days after the onset of clinical symptoms and IgG at 8-13 days after SARS-CoV-2 virus infection.¹³

Researcher's opinion, examining IgM and IgG antibodies is reliable and valuable because of the relatively fast processing time. In patients presenting with discrepancies between clinical/radiological features and molecular testing, rapid antibody detection may be an additional element that helps clinicians make correct diagnoses. However, it is recommended that this test be done on the first day the symptoms appear.

CONCLUSIONS AND SUGGESTIONS

IgG has higher sensitivity than IgM, while IgM has higher specificity, positive predictive value, and negative predictive value than IgG. IgM and IgG had the same positive predictive value, negative predictive value, and efficiency value. Future studies should evaluate the SARS-CoV-2 IgM and IgG antibody rapid diagnostic tests with the seroconversion assessment of positive COVID-19 patients and determine other diseases in non-COVID-19 patients.

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