INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 156
2022 March; 28(2): 156 -160
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

# Validity ICT and CLIA of SARS-CoV-2 Antibody

**Fili Oei, Asvin Nurulita, Darwati Muhadi, Mansyur Arif**

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Central General Hospital, Makassar, Indonesia. E-mail: filioei@live.com

**ABSTRACT**

The varying validity of antibody tests requires a good diagnostic accuracy analysis in determining the right time to perform antibody testing in patients. This study aimed to determine the sensitivity and specificity of the SARS-CoV-2 antibody test based on the testing time. This study was a cross-sectional retrospective diagnostic test study involving 960 subjects divided into three groups based on the testing time using two methods of ICT and CLIA. The sensitivity and specificity of ICT method were 45.8% and 59.7%, while those of the CLIA method were 81.6% and 74.4%, respectively. Based on the timing of antibody test post-symptom onset, the highest sensitivity was obtained at >15 days post-symptom onset (84.4% and 78.7%). Low sensitivity might be caused by the absence or low antibodies in the first week to second weeks of infection. Sample collection was performed at 2-4 weeks post-symptom onset. The SARS-CoV-2 IgM and IgG antibodies testing showed high sensitivity and specificity (84.4% and 78.7%) at >15 days post-symptom onset. In addition, antibody testing using the CLIA method showed higher sensitivity and specificity compared to the ICT method.

**Keywords:** COVID-19, antibody, sensitivity, specificity

## INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) virus. The SARS-CoV-2 virus is a single-stranded RNA (ssRNA) virus belonging to the Betacoronavirus genus in the Coronaviridae family. The transmission of the SARS-CoV-2 virus is via droplets and direct contact with an incubation period of 14 days. Symptoms are fever, cough, fatigue, anorexia, respiratory problems, and headaches.[1]

According to the World Health Organization (WHO) on October 17, 2020, there were a total of 39,633,171 positive cases and 1,109,834 (2.8%) COVID-19 death cases worldwide. Indonesia ranks 18$^{th}$ in the world with 357,762 confirmed positive cases and 12,431 (3.48%) death cases by October 17, 2020. In addition, South Sulawesi ranks fifth in Indonesia with 17,346 positive cases and 442 (2.55%) deaths.[2]

SARS-CoV-2 antibody tests are divided into serological tests to detect SARS-CoV-2 antibodies and molecular tests to detect viral RNA. The method of serological tests is Immunochromatography (ICT), Enzyme-Linked Immunosorbent Assay (ELISA), and Chemiluminescence Immunoassay (CLIA). The molecular test method available is a rapid molecular test and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) has been widely used to diagnose COVID-19 and remains the gold standard by WHO. Serologic testing can be used as a potential screening test for individuals at risk in the future; however, it is not recommended as a diagnostic test for COVID-19.[1]

A study by Bastos *et al*. showed that the ICT method had low sensitivity of 66.0% (Confidence Interval/CI 49.3%-79.3%), while that of the CLIA method was 97.8% (46.2%-100%), with a specificity of 96.6%-99.7%. Another study by Vengesai *et al.* showed that the sensitivity of the ICT and CLIA method was 58.6% (CI 46.37%-68.86%) while that of the CLIA method was 91% (85.16%-93.11%) and its specificity was between 96.93%-99.91%. Meanwhile, research by Xie *et al*. showed that the ICT method has a higher sensitivity (88.6%), with a specificity of 90.63% and those of the CLIA method were 86.9% and 99.2%, respectively.[3-5]

The study by Wang *et al*. showed that serologic tests had low sensitivity at 0-7 days of onset (< 40%) and highest at >14 days. The study of Whitman *et al*., showed that the sensitivity of the test increased along with time, the sensitivity on day 1-5 was 40%, days 6-10 was 66.67%, days 11-15 was 81.82), days 16-20 was 80.95%, and >20 days was 81.82%.[6,7]

Among the all SARS-CoV-2 antibody testing in Indonesia, there are 63 types of RDT used in all

laboratories in Indonesia with various samples. Sampling that can be done for antibody examination is capillary blood, serum, plasma, and whole blood. The sensitivity of the SARS-CoV-2 IgG test varies from 33 to 96% and specificity of 10-100%, while the sensitivity and specificity of the SARS-CoV-2 IgM test vary from 16 to 100% and from 7 to 97%, respectively.[8]

COVID-19 is a new disease that has become a world pandemic. This disease should be carefully monitored due to its relatively rapid transmission, high mortality rate, and unknown definitive therapy. Serologic tests are widely available, and the analysis of their diagnostic accuracies such as sensitivity and specificity is needed to determine the right time to perform antibody testing in patients. This study aimed to determine the sensitivity and specificity of the SARS-CoV-2 antibody testing based on the ICT and CLIA method and the day of post-symptom onset.

## METHODS

This study was a diagnostic test with a cross-sectional retrospective design conducted at Dr. Wahidin Sudirohusodo Central General Hospital, Makassar from April to August 2020. The subject was randomized and that filled the inclusion and exclusion criteria. The inclusion criteria were participants > 18 years old and all patients tested for both antibody test and RT-PCR of SARS-CoV-2. The exclusion criteria were immunocompromised patients (such as neoplasms, autoimmune disease, HIV-AIDS), patients with pregnancy, age < 1 year, and those who did not perform any antibody and RT-PCR of SARS-CoV-2. The antibody tested in this study were IgM and IgG antibodies by using serum samples. Detection of anti-SARS-CoV-2 antibody was performed with two serologic assays using the ICT method and five brand reagent groups (Acon, Zybio, Standard Q, Standard Q Combo, and Zhuhai Lamfeng) and CLIA method using one reagent group (AFIAS COVID-19 Ab). The results of this study were also divided into three groups based on the length of antibody testing since post-symptoms onsets, such as <7 days, 8-15 days, and > 15 days.

The diagnosis of COVID-19 using RT-PCR (LightCycler 480 II, Roche) was performed with the ORF-1ab gene and N gene as the target genes. The AFIAS COVID-19 Ab assay is a sandwich CLIA for the determination of IgG and IgM antibodies against SARS-CoV-2 using recombinant nucleocapsid protein as antigen. The ICT assay is a lateral flow assay for the determination of IgG and IgM antibodies.

The data analysis was performed by using SPSS Statistics (IBM). The ethical clearance of this study was obtained from the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Central General Hospital, Makassar with article number 527/UN4.6.4.5.31/PP36/2020.

## RESULTS AND DISCUSSIONS

This study was carried out on 960 subjects who met the inclusion criteria such as patients whose complete laboratory test results of the COVID-19 IgM, IgG, and RT-PCR test. A total of 40 subjects were excluded because their age was less than 2 years old. The research subjects in this study consisted of 414 male and 546 female subjects. The antibody testing based on post-symptoms onset in this study were as follows: Test at <7 days were reported from 694 subjects (72.3%); Test at day 8-15 were reported from 180 subjects (18.8%) and; Test at >15 days were reported from 98 (9.0%) subjects. This study found reactive antibody test results in 265 (27.6%) subjects, non-reactive results in 401 (41.8%) subjects, and combined test results of IgG and IgM in 294 (30.6%)

**Table 1.** Characteristics of research subjects

| | | Total (n) | % |
|---|---|---|---|
| **Gender** | Male | 414 | 43.1 |
| | Female | 546 | 56.9 |
| **Post-symptoms onset** | < 7 days | 694 | 72.3 |
| | 8-15 days | 180 | 18.8 |
| | > 15 days | 86 | 9.0 |
| **Antibody test** | Reactive | 265 | 27.6 |
| | Non-reactive | 401 | 41.8 |
| | Combination | 294 | 30.6 |
| **RT-PCR test** | Positive | 493 | 51.4 |
| | Negative | 467 | 48.6 |

subjects. In addition, positive and negative RT-PCR test results were found in 493 (51.4%) and 467 (48.6%) subjects, respectively.

Table 1 describes the characteristics of the research subjects based on gender, testing time based on the post-symptom onset, antibody test results, and RT-PCR test results.

Table 2 shows the diagnostic value of the SARS-CoV-2 IgM-IgG antibody test based on the reagent groups with low sensitivity and high specificity values. The highest sensitivity was found in reagent groups 1 and 6 (81.6% and 75%), and the lowest was found in group 4 (20.4%). However, these reagent groups showed high specificity with a range of 74.7%-92.3%.

Table 3 shows the comparison of the IgM-IgG antibody test between the ICT and CLIA methods. The ICT method showed low sensitivity and specificity (45.8% and 59.7%), while the CLIA method showed high sensitivity and specificity (81.6% and 74.7%).

Table 4 shows the diagnostic value of the SARS-CoV-2 IgM-IgG antibody test for each reagent group based on the testing time of post-symptom onset with the lowest sensitivity value at the examination time <15 days and the highest sensitivity at >15 days. Reagents in group 6 showed high sensitivity but low specificity. However, reagents in group 1 showed high sensitivity and specificity at the testing time of >15 days.

Table 5 shows a comparison between the ICT and CLIA methods based on the antibody testing time of post-symptom onset. The ICT method showed low sensitivity and specificity at <7 days, 8-15 days, or >15 days, while the CLIA method showed the highest

**Table 2.** Diagnostic value of SARS-CoV-2 IgM-IgG antibody test based on reagent groups

| | **AFIAS** | | **Acon** | | **Zybio** | | **Standard Q** | | **Standard Q Combo** | | **Zhuhai Lamfeng** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % | n | % |
| Sensitivity | 266 | 81.6 | 47 | 35.5 | 40 | 22.5 | 48 | 20.4 | 48 | 31.3 | 44 | 75.0 |
| Specificity | 194 | 74.7 | 53 | 90.6 | 60 | 91.7 | 52 | 92.3 | 52 | 78.8 | 56 | 82.1 |

**Table 3.** Comparative diagnostic value of IgM-IgG antibody test between the ICT and CLIA methods

| | **Total (n)** | **ICT (%)** | **Total (n)** | **CLIA (%)** |
|---|---|---|---|---|
| Sensitivity | 227 | 45.8 | 266 | 81.6 |
| Specificity | 273 | 59.7 | 194 | 74.7 |

**Table 4.** Diagnostic value of SARS-CoV-2 IgM-IgG antibody test for each reagent group based on the testing time of post-symptom onset

| | **< 7 days** | | | | **8-15 days** | | | | **> 15 days** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitivity | | Specificity | | Sensitivity | | Specificity | | Sensitivity | | Specificity | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| Afias | 101 | 40.6 | 125 | 52.8 | 101 | 50.5 | 22 | 68.2 | 64 | 84.4 | 47 | 78.7 |
| Acon | 39 | 38.5 | 36 | 30.6 | 6 | 33.3 | 5 | 60.0 | 4 | 50.0 | 10 | 50.0 |
| Zybio | 30 | 30.0 | 34 | 70.6 | 6 | 33.3 | 11 | 72.7 | 4 | 50.0 | 15 | 73.3 |
| Standard Q | 32 | 46.9 | 37 | 59.5 | 15 | 46.2 | 11 | 72.7 | 2 | 50.0 | 3 | 66.7 |
| Standard Q Combo | 32 | 40.6 | 45 | 66.7 | 14 | 50.0 | 4 | 50.0 | 2 | 50.0 | 3 | 66.7 |
| Zhuhai Lamfeng | 17 | 64.7 | 45 | 64.4 | 13 | 76.9 | 5 | 60.0 | 14 | 71.4 | 6 | 50.0 |

**Table 5.** A diagnostic value between the ICT dan CLIA method based on the antibody testing time of post-symptom onset

| | **< 7 days** | | | | **8-15 days** | | | | **> 15 days** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ICT | | CLIA | | ICT | | CLIA | | ICT | | CLIA | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| Sensitivity | 150 | 32.0 | 101 | 40.6 | 54 | 37.0 | 101 | 50.5 | 23 | 65.2 | 64 | 84.4 |
| Specificity | 197 | 50.8 | 125 | 52.8 | 36 | 50.0 | 22 | 68.2 | 40 | 60.0 | 47 | 78.7 |

sensitivity and specificity at >15 days (84.4% and 78.7%).

The results of the SARS-CoV-2 antibody test based on the reagent group (Table 2) showed that groups 1 (Afias) and 6 (Zhuhai Lamfeng) had high sensitivity and specificity (81.6%-74.7% and 75%-82.1%). Meanwhile, the other group showed a low sensitivity with a range of 20.4%-35.5%, but high specificity with a range of 74.7%-92.3%. In addition, based on the method of antibody test used (Table 3), it was shown that the CLIA method has high sensitivity and specificity (81.6% and 74.7%) compared to the ICT method with low sensitivity and specificity (45.8% and 59.7%).

These results were consistent with research by Bastos *et al*., which showed that the ICT method had lower sensitivity than the CLIA method. However, the results of this study were different from the research of Xie *et al*., showing that the sensitivity to the ICT method was higher than the CLIA method. However, although CLIA has high sensitivity and specificity, antibody-based serologic testing is not recommended by WHO for diagnosis of COVID-19 but rather is used for screening to assist the surveillance and epidemiology of the disease.[9]

The sample was tested at a different time for each patient. The results of the SARS-CoV-2 IgM-IgG antibody test based on post-symptom onset (Table 4) showed a low sensitivity value at the <15 days post-symptom onset and the highest sensitivity at >15 days. Group 6 showed high sensitivity but low specificity. Meanwhile, group 1 showed high sensitivity and specificity at the testing time >15 days. Contrastingly, group 3 showed low sensitivity but high specificity from the testing time at <7 days to 15 days.

The results of antibody test using the ICT and CLIA methods based on the length of time of examination post-symptom onset (Table 5) showed that using the CLIA method had high sensitivity and specificity (84.4% and 78.7%) were obtained at the time of examination >15 days compared to using the ICT method (65.2% and 60%). Whereas at the time of examination <15 days, the sensitivity and specificity were low using both the CLIA and ICT methods.

The results of this study were in line with the study by Wang *et al*., and Whitman *et al*. suggesting that the sensitivity of the test increased over time with high sensitivity and specificity values at the testing time of >15 days of post-symptom onset compared to <7 days with low sensitivity and specificity.[6,7]

The differences in sensitivity and specificity results in this study might be caused by several factors such as age, nutritional status, disease severity, and the presence of other infections. In addition, it might also be caused by the absence of antibodies from the first week to the second week of infection. Cross-reaction with other types of Coronaviruses and Dengue virus can also occur during the detection of SARS-CoV-2 antibody testing that it can cause false-positive or false-negative results.[9]

The measurement of specific antibody responses, which may take some weeks to develop after disease onset may reduce the sensitivities of the assays. If blood samples were collected during the early stage of the infection, false-negative results may be obtained. This is because the test does not directly detect the presence of the virus. However, antibodies may be detected when SARS-CoV-2 is no longer present, giving false-positive results.[9]

The factors that can influence false-negative results are the absence of antibodies at the time of sampling (during the incubation period or window period), low antibody levels or antibody levels below the reagents detection limit, no response of antigens to antibodies causing the coverage of epitope by other components that it cannot bind to antibodies, errors in the pre-analytic, analytical, and post-analytic stages. In addition, cross-reaction with other types of viral antibodies may lead to false-positive results.

The cross-reaction between SARS-CoV-2 and Dengue virus can be caused by the binding between the Receptor Binding Motif (RBM) of the SARS-CoV-2 spike protein and the angiotensin-converting enzyme-2 (ACE-2) receptor. In addition to the cross-reaction with the Dengue virus, cross-reactions with other types of Flavivirus such as Zika virus and Japanese encephalitis may also occur. SARS-CoV-2 and SARS-CoV are composed of 90% amino acid consisting of N protein and 77% of S protein, while SARS-CoV-2 and MERS-CoV consist of 49% amino acid of N protein and 33% of S protein. The composition of these amino acids can cause cross-reactions.[10]

World Health Organization recommends that sample collection be carried out at 2-4 weeks post-symptom onset. The low antibody, slow response of antibodies, and especially the sampling time when antibodies have not been formed may explain the low ability of the test to detect antibodies. The IgM antibody is formed on days 5-12 and slowly decreases since then, while IgG peaks after day 20 or after the disappearance of IgM. This might cause the low sensitivity at < 7 days and sensitivity of 100% at >15 days.[11,12]

## CONCLUSIONS AND SUGGESTIONS

There were several limitations to this study. First, the different testing times because of the social distancing and the different number of research subjects involved in both methods. Second, there was no observation of the possible cross-reactivity for either IgM or IgG in research subjects. Third, there was no evaluation of positive and negative predictive value and the impact of prevalence in this study. Lastly, there was no evaluation of the seroconversion time and seronegativity of SARS-CoV-2 IgM and IgG among patients.

The antibody testing of SARS-CoV-2 with ICT methods showed lower sensitivity and specificity (45.8% and 59.7%) compared to the CLIA method with high sensitivity and high specificity (81.6% and 74.7%). Based on the test time, SARS-CoV-2 IgM and IgG antibodies showed higher sensitivity and specificity at >15 days post-symptom onset compared to <7 days and 8-15 days post-symptom onset. The test time >15 days by using the CLIA method has high sensitivity and specificity (84.4% and 78.7%) compared to the ICT method (65.2% and 60%). The ICT method had low sensitivity and specificity than the CLIA method, but it provides a fast turnaround time and can be used for emergency cases.

Both ICT and CLIA could play an important role in the diagnosis of suspected COVID-19 infections and also could contribute to the understanding of the immunological state of the population. Further studies assessing antibody assays against gene profiles based on the length of time of testing may be suggested to assess the accuracy of serologic assays.

## REFERENCES


1. Aryati. Aspek laboratorium COVID-19: Pemeriksaaan serologi dan PCR. Seminar PDS PatKLIn pada 21 Mei 2020.
2. Anonim. Coronavirus Diseases (COVID-19) pandemic. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed October 17, 2020).
3. Bastos ML, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, *et al.* Diagnostic accuracy of serological test for COVID-19: Systematic review and meta-analysis. BMJ, 2020; 370. Available from: https://www.bmj.com/content/370/bmj.m2516. (accessed October 14, 2020).
4. Vengesai A, Midzi H, Kasambala M, Mutandadzi H, Mduluza-Jokonya TL, *et al.* A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19. Research Square, 2020; 1-41. Available from: https://doi.org/10.21203/rs.3.rs-34638/v1 (accessed October 14, 2020).
5. Xia N, Wang G, Gong W. Serological test is an efficient supplement of RNA detection for confirmation of SARS-CoV-2 infection. Preprints, 2020; 1-6 (accessed October 14, 2020).
6. Wang H, Ai J, Loeffelholz MJ, Yang YW, Zhang W. Meta-analysis of diagnostic performance of serology test for COVID-19: Impact of assay design and post-symptom-onset intervals. Emerging Microbes & Infections, 2020; 9(1): 2200-2211.
7. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, *et al.* Test performance evaluation of SARS CoV-2 serological assays. Infectious disease, 2020. medRxiv, 2020; 2020.04.25.20074856. Available from: http://medrxiv.org/lookup/doi/10/1101/2020.04.25.20074856 (accessed October 14, 2020).
8. PP PDS PatKLIn. Hasil survey performa RDT antibodi SARS CoV-2. Juli 2020. Available from: http://pdspatklin.or.id/post/performa-rdt-sars-cov-2-pdspatklin. (accessed October 17, 2020).
9. World Health Organization (WHO). Advice on the use of point-of-care immunodiagnostic test for COVID-19. Available from: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19 (accessed October 14, 2020).
10. Nath H, Mallick A, Roy S, Sukla S, Basu K, *et al.* Dengue antibodies can cross-react with SARS-CoV-2 and vice versa-antibody detection kits can give false-positive results for both viruses in regions where both COVID-19 and Dengue co-exist. medRxiv, 2020; 2020.07.03.20145797. Available from: https://doi.org/10.1101/2020.07.03.20145797 (accessed October 17, 2020).
11. de Moura DTM, McCarty TR, Ribeiro IB, Funari MP, de Oliviveria PVAG, *et al.* Diagnostic characteristics of serological-based COVID-19: A systematic review and meta-analysis. Clinics (Sao Paulo). 2020; 75:e2212. Available from: https://doi.org/10.6061/clinics/2020/e2212 (accessed October 14, 2020).
12. Infectious Disease Society America guidelines on the diagnosis of COVID-19: Serologic testing. Available from: https://www.idsociety.org/ COVID19 guidelines/serology (accessed October 14, 2020).