

Shun Kaneko ORCID iD: 0000-0003-2660-6580

Clinical validation of an immunochromatographic SARS-Cov-2 IgM/IgG antibody assay with Japanese cohort

Short running title: Clinical validation of SARS-Cov-2
IgM/IgG antibody

Shun Kaneko¹, Yoko Nukui², Takeshi Arashiro², Yosibumi Aiso², Maya Sugii²,
Yoshiro Hadano², Kaoru Nagata³, Reiko Taki⁴, Ken Ueda³, Satoko Hanada⁴,
Shinichiro Suzaki⁵, Naoshige Harada⁵, Yoshimi Yamaguchi⁶, Hiroyuki Nakanishi¹,
Masayuki Kurosaki¹, Masayuki Nagasawa⁷, Namiki Izumi¹

1 Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital,
Tokyo, Japan

2 Department of Infection Control and Prevention, Tokyo Medical and Dental
University Medical Hospital, Tokyo, Japan

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3 Department of General Medicine, Musashino Red Cross Hospital, Tokyo, Japan

4 Department of Pulmonary Medicine, Musashino Red Cross Hospital, Tokyo,
Japan

5 Department of Emergency and Intensive Care, Musashino Red Cross Hospital,
Tokyo, Japan

6 Division of Clinical laboratory, Musashino Red Cross Hospital, Tokyo, Japan

7 Division of Infection Control and Prevention, Musashino Red Cross
Hospital, Tokyo, Japan

Contact information

Address reprint requests to: Namiki Izumi, M.D., Ph.D.,

Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital

1-26-1, Kyonan-cho, Musashino-shi Tokyo 180-8610, JAPAN.

Tel: +81-422-32-3111. Fax: +81-422-32-9551.

E-mail: izumi012@musashino.jrc.or.jp (N.Izumi)

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The authors declare that they have no conflicts of interests.

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The Ethical Committee of Musashino Red Cross Hospital, and Tokyo Medical and Dental University Medical Hospital approved this study, which was conducted in accordance with the Declaration of Helsinki (confirmation number:2020-444, M2020-029, respectively).

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Author contribution statement

Study concept and design: S.K, Y.N, M.N, and N.I. Acquisition of data: T.A, Y.A, M.S, Y.H, K.N, R.T, K.U, S.H, S.S, N.H, Y.Y, H.N, M.K and M.N. Analysis and interpretation of data: S.K, Y.N, M.N and N.I. Drafting of the manuscript: S.K, Y.N, and N.I. Statistical analysis: S.K, Y.N and M.N. Study supervision: N.I. Final approval: All of the authors. Agreement to be accountable for all aspects of the work: All of the authors.

Abstract

Background: The coronavirus disease 2019 (COVID-19) has become a major health threat. To overcome COVID-19, appropriate diagnosis methods are urgently needed. The aim of this study was to clinically evaluate the colloidal gold immunochromatography assay for SARS-Cov-2 IgM/IgG antibody (Ab).

Methods: Patients confirmed COVID-19 (n = 51) were recruited prospectively from the Musashino Red Cross hospital and Tokyo Medical and Dental University Medical Hospital, between March and May 2020. And the analytical specificity was assessed with serum samples of patients without COVID-19 (n=100) collected

between August to September 2019 before SARS-CoV-2 was first reported in China.

Results: Among COVID-19 patients, a total of 87 serum samples were tested for SARS-Cov-2 IgM/IgG Ab assay. IgM was detected 71.0 %, 86.9 %, and 83.3 % at day8-14, 15-28, >29 after symptom onset and IgG was detected in 81.6 %, 87.0 %, and 94.4 %, respectively. The sensitivity of IgM and IgG Ab after day8 assay was significantly higher than before day7, respectively ($p=0.0016$, 0.0003). There were no positive results in 100 serum samples from patients without COVID-19.

Conclusion: The SARS-Cov-2 IgM/IgG Ab assay had 79.7% / 86.1% sensitivity (the 8 days after from onset) and 100% specificity in this population.

Key words

COVID-19, colloidal gold immunochromatography assay, SARS-Cov-2 IgM/IgG antibody

Introduction

The coronavirus disease 2019 (COVID-19) was firstly reported in Wuhan, China in December 2019, and the outbreak of COVID-19 had become a big threat to not

only China but also all over the world. As of June 8, 2020, more than 6.93 million cases had COVID-19 and more than 400,000 deaths had been reported globally¹.

Coronaviruses (CoV) are a large family of viruses that cause illness from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). SARS-CoV-2, the cause of COVID-19 is a new strain that has not been previously identified in humans.²

Common COVID-19 includes respiratory symptoms, fever, shortness of breath and breathing difficulties.³ In more severe cases, infection causes pneumonia, severe acute respiratory syndrome, kidney failure and even death. Based on systematic review and meta-analysis, older patients were identified to be at a fatal disease course, with the most prevalent comorbidities of hypertension and diabetes which were associated with the severity of COVID-19^{4,5}. Acute respiratory distress syndrome (ARDS) and acute cardiac injury may be the main obstacles for patients to treatment recovery⁶.

To establish diagnosis of COVID-19, quantitative reverse-transcription polymerase chain reaction (RT-PCR) analysis is considered the current standard. However, it requires special equipment, a time-consuming protocol, and laboratory

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technicians. The most important points are RT-PCR requires samples from the upper and lower respiratory tract, the process of collecting samples and extracting RNA increases the risk of exposure to the viral droplet. The false-negative results for diagnosing COVID-19 were sometimes observed because adequate sampling from nose or throat was difficult for some patients.

Current estimates of the incubation period ranged from 1-12.5 days with median estimates of 5-6 days^{3,7}. Based on information from other coronavirus diseases, such as MERS and SARS, the incubation period of SARS-CoV-2 could be up to 14 days.

Plasma anti-SARS-CoV-2 antibody (Ab) was firstly reported in the convalescent plasma in five patients who recovered from the SARS-CoV-2 infection⁸. Recently, several commercial assays that detect IgM/IgG Ab against SARS-CoV2 have become available for use in the clinical setting^{9,10}. However, their clinical usefulness has yet to be thoroughly evaluated. Moreover, it is important to evaluate the number of the patients infected with COVID-19 in specific area and to establish the strategy to prevent the spread of viral infection for the specific population.

In the present study, we analyzed the diagnostic accuracy of SARS-Cov-2 IgM/IgG Ab assay; 2019-nCoV Ab Test Cassette (Colloidal Gold) (INNOVITA, Beijing, China). Samples were collected from the patients with acute COVID-19 infection confirmed by RT-PCR and non-infected patients admitted by other disease in August 2019, before the spread of COVID-19 infection.

Methods

1. Samples

Positive samples from patients with COVID-19

51 patients with acute COVID-19 infection who were admitted to Musashino Red Cross Hospital and Tokyo Medical and Dental University Medical Hospital, between March and May 2020 were included in this study. A total of 87 serum samples were tested for SARS-Cov-2 IgM/IgG Ab assay. All the patients were examined by RT-PCR for SARS-CoV2 using pharyngeal and nasopharyngeal swabs collected at public health institute or hospitals in accordance with the nationally recommended method in Japan¹¹. Clinical information was collected from the medical records.

Negative samples from patients with non-COVID-19

The analytical specificity of the SARS-Cov-2 IgM/IgG Ab assay was assessed using serum samples that were collected between August to September 2019 from the patients admitted before SARS-CoV2 was first reported in China. These serum samples (n=100) were collected from several diseases such as hepatitis C virus infection and so on in Musashino Red Cross hospital and Tokyo Medical and Dental University Medical Hospital, which served as negative controls. Clinical information was collected from medical records, and all serum samples were stored at -80°C before use in the assay.

2. Serum antibody assay

Serum SARS-Cov-2 IgM/IgG Ab assay was analyzed using 2019-nCoV Ab Test Cassette (Colloidal Gold) (INNOVITA, Beijing, China) according to the manufacturer's protocol. In brief, 10 µL serum/plasma or 20uL whole blood was added to the sample well. Subsequently, 80uL (2drops) of sample buffer was added to the same sample well, and the results were interpreted within 15 minutes of incubation. The presence of only the control (C) line indicated a negative result; the

presence of both the control line (C) and the IgM or IgG antibody (T) line indicated a positive result for IgM or IgG Ab, respectively.

3. Definition of the days from onset

All of the patients included in the present study had clinical symptoms such as fever, cough, diarrhea, malaise, and/or tachypnea. We defined the days collecting serum Ab samples after the onset of the first symptoms.

4. Statistical analysis

The Chi-squared and Fisher's exact tests were used to compare categorical variables. Significance was set at $P < 0.05$. The GraphPad Prism software (GraphPad Software, San Diego, CA, USA) was used to analyze statistical significance.

5. Ethical statement

This study was approved by the Ethical Committee of Musashino Red Cross Hospital, and Tokyo Medical and Dental University Medical Hospital that was conducted in accordance with the Declaration of Helsinki (confirmation number: 2020-444, M2020-029, respectively).

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Results

In 51 confirmed COVID-19 cases, the median age was 63 (25-95) years old, 37 patients were male (72.5%). There were no asymptomatic patients with COVID-19.

Total 87 serum samples were tested for IgM/IgG COVID-19 Ab assay.

As shown in Figure1, the sensitivity was dependent on the days from onset.

IgM Ab were detected 0%, 16.7%, 71.0%, 86.9% and 83.3% at 0-4days, 4-7days, 8-14days, 15-28days and >29days after symptom onset, while IgG Ab were detected 0%, 16.7%, 81.6%, 87.0% and 94.4%, respectively.

The sensitivity of SARS-Cov-2 IgM/IgG Ab assay after >8 days from onset was significantly higher than before 7th day, respectively ($p=0.0016$, 0.0003 , Chi-square test).

To investigate specificity, we tested for negative control serum samples collected from August to September in 2019 from the patients admitted before SARS-CoV2 was first reported in China. As results, there were no positive results in 100 patients' samples. This assay achieved 100% specificity.

The SARS-Cov-2 IgM/IgG Ab assay had 79.7% (63/79) / 86.1% (68/79) sensitivity (after >8 days from onset) and 100% specificity.

Discussion

This study provides evidence that SARS-Cov-2 IgM/IgG Ab assay is useful for patients based on Japanese cohort in a real-world clinical setting.

Though the RT-PCR for upper respiratory specimens have been standard test for COVID-19, it sometimes had limitations due to technical materials, exposure risk of sampling and taking time to result. The time lapse from the onset affects the sensitivity of tests and may cause false negative results. Some reports suggest that sensitivity of RT-PCR for upper respiratory specimens got decreased after about 10 days from onset^{12,13}. On the other hand, it is considered that the disease becomes severe within about 1 week after the onset⁷. In more severe cases, it seems that the process of admission to the intensive care unit will be followed after 10days.^{7,14} These findings suggest that diagnosis with RT-PCR alone may overlook severe COVID-19 cases.

Recently, SARS-Cov-2 IgM/IgG Ab evaluated by enzyme-linked immunosorbent assay (ELISA) based on the recombinant nucleocapsid protein of SARS-CoV-2 were detected in the serum as early as the 4 days after symptom onset. The sensitivity and specificity of IgM Ab was reported as 77.3%, and 100%, while it was 83.3% and 95.0% for IgG Ab, respectively⁹. More recently, Imai et al. reported that IgM Ab were detected in 27.8 %, 48.0 %, and 95.8 % of the specimens collected within 1 week, 1-2 weeks, and >2 weeks after symptom onset, while IgG Ab were detected in 3.3%, 8.0%, and 62.5%, respectively¹⁰. From these results, they concluded that IgM/IgG Ab assay can be detected in the middle and later stage of the illness, suggesting an important role in the diagnosis of COVID-19 as a complement approach for viral nucleic acid assays.

In this study, the SARS-Cov-2 IgM/IgG Ab assay had 79.7% (63/79) / 86.1% (68/79) sensitivity (after >8 days from onset) and 100% specificity (Figure.1). This result supports these reports^{9,10}.

The present study had several limitations. First, although the study showed the usefulness of the SARS-Cov-2 IgM/IgG Ab assay as a clinical setting report, this result was in Japanese populations. They may not be able to adapt to racial

differences. The world-wide reports and validation studies are expected. Second, the observation period and number of patients may be insufficient for solid comparisons. It is even better if there are RT-PCR results at the same timing or comparison of samples derived from the same patients at time dependent manner. Although there are reports that they do not have cross-antibodies with other coronaviruses, we have not actually confirmed cross-antibodies in this study. Finally, SARS-Cov-2 IgM/IgG Ab assay was not complete blind test. It might cause a subjective tendency, although test was performed at once for much samples including stored samples as possible not to affect bias.

In conclusion, SARS-Cov-2 IgM/IgG Ab assay has 100% specificity and is useful for COVID-19 detection especially after 8days from onset. This assay will be widely accepted as an easy to perform, rapid, and high throughput method. Combination SARS-Cov-2 IgM/IgG antibody assay and RT-PCR may contribute appropriate diagnosis of COVID-19.

Data Availability Statement

Data available on request due to privacy/ethical restrictions.

References

1. World Health Organization. Coronavirus disease (COVID-19). Situation Report–
140. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200608-covid-19-sitrep-140.pdf?sfvrsn=2f310900_2 (accessed, June 9, 2020)
2. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020;382(8):727-733.
3. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* 2020;382(18):1708-1720.
4. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054-1062.
5. Zheng Z, Peng F, Xu B, et al. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect.* 2020.

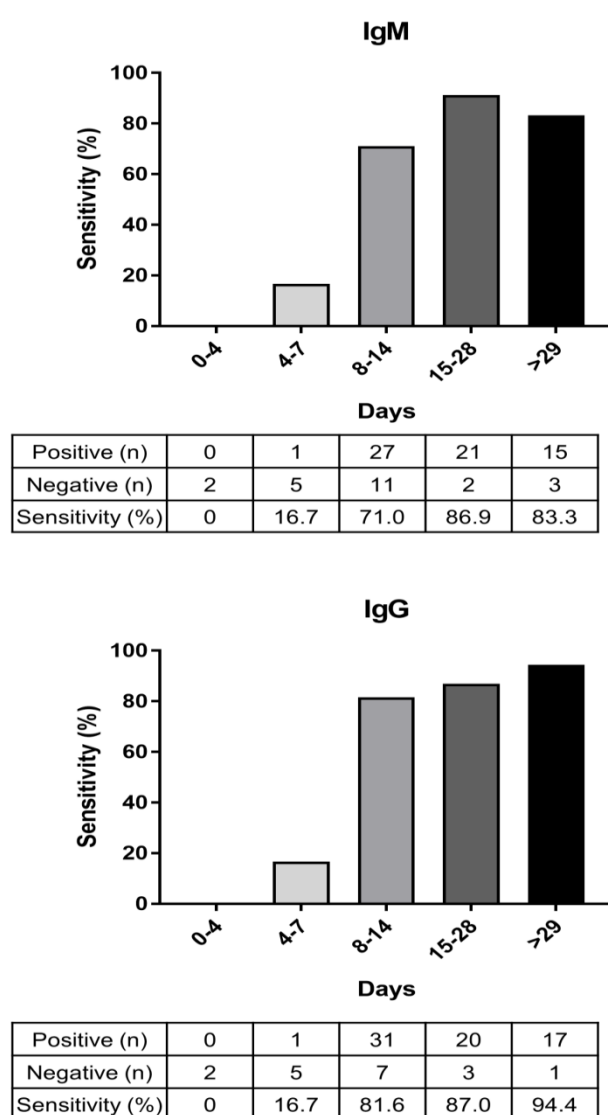
6. Hu Y, Sun J, Dai Z, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Virol.* 2020;127:104371.
7. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N Engl J Med.* 2020;382(13):1199-1207.
8. Zhang L, Pang R, Xue X, et al. Anti-SARS-CoV-2 virus antibody levels in convalescent plasma of six donors who have recovered from COVID-19. *Aging (Albany NY).* 2020;12(8):6536-6542.
9. Xiang F, Wang X, He X, et al. Antibody Detection and Dynamic Characteristics in Patients with COVID-19. *Clin Infect Dis.* 2020.
10. Imai K, Tabata S, Ikeda M, et al. Clinical evaluation of an immunochromatographic IgM/IgG antibody assay and chest computed tomography for the diagnosis of COVID-19. *J Clin Virol.* 2020;128:104393.

11. Shirato K, Nao N, Katano H, et al. Development of Genetic Diagnostic Methods for Novel Coronavirus 2019 (nCoV-2019) in Japan. *Jpn J Infect Dis.* 2020.
12. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis.* 2020;20(4):411-412.
13. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. In: *N Engl J Med.* Vol 382.2020:1177-1179.
14. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.

Figure Legends

Figure 1. SARS-Cov-2 IgM/IgG Ab assay with serum samples of COVID-19

positive patients.



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