



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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## Case Report

## False positive results in severe acute respiratory coronavirus 2 (SARS-CoV-2) rapid antigen tests for inpatients



Kazuhiro Itoh <sup>a, b, \*</sup>, Toru Kawamitsu <sup>c</sup>, Yoko Osaka <sup>c</sup>, Kazuyo Sato <sup>c</sup>, Yusuke Suzuki <sup>b</sup>, Chiyo Kiriba <sup>b</sup>, Yumi Saito <sup>d</sup>, Ryo Hirose <sup>d</sup>, Hiroshi Ichihara <sup>d</sup>, Mitsugu Saito <sup>a</sup>, Yasuhiko Mitsuke <sup>b</sup>, Kenichi Kuzumi <sup>e</sup>, Hirofumi Miyashita <sup>f</sup>, Hiroshi Tsutani <sup>b</sup>

<sup>a</sup> Infection Defense Committee, National Hospital Organization Awara Hospital, Awara, Japan

<sup>b</sup> Department of Internal Medicine, National Hospital Organization Awara Hospital, Awara, Japan

<sup>c</sup> Department of Pediatrics, National Hospital Organization Awara Hospital, Awara, Japan

<sup>d</sup> Department of Laboratory Medicine, National Hospital Organization Awara Hospital, Awara, Japan

<sup>e</sup> Sakai Health and Welfare Center, Fukui Prefectural Government, Awara, Japan

<sup>f</sup> Health Promotion and Disease Prevention Division, Department of Health and Welfare, Fukui Prefectural Government, Fukui, Japan

## ARTICLE INFO

## Article history:

Received 3 December 2020

Received in revised form

5 March 2021

Accepted 14 March 2021

Available online 22 March 2021

## Keywords:

Rapid antigen detection test

SARS-CoV-2

COVID-19

## ABSTRACT

Severe acute respiratory syndrome coronavirus 2 rapid antigen detection (RAD) test kits are widely used as primary screening test in Japan because rapid diagnosis of coronavirus disease 2019 (COVID-19) is critical for infection control. We report cases with RAD test false-positive results in a ward for patients with disabilities. RAD tests potentially evoke hospital operational risk. It is desirable that performing PCR test appropriately when patients admitted to a medical treatment ward with COVID-19 symptoms instead of RAD test.

© 2021 Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus that causes coronavirus disease 2019 (COVID-19) infection, has recently emerged and caused a pandemic. COVID-19 outbreaks in hospitals or welfare facilities have become a significant social problem, such as the aggravation of inpatients and residents at risk of underlying diseases and old age and the difficulty of securing human resources because of staff infection [1–3]. In Japan, rapid antigen detection (RAD) test kits based on the immunochromatography method are widely used as primary screening test because early detection of COVID-19 is important for institutional infection control. We very recently experienced false-positive cases detected by RAD tests used for febrile patients hospitalized in the ward of persons with disabilities (see Fig. 1).

## 2. Case report

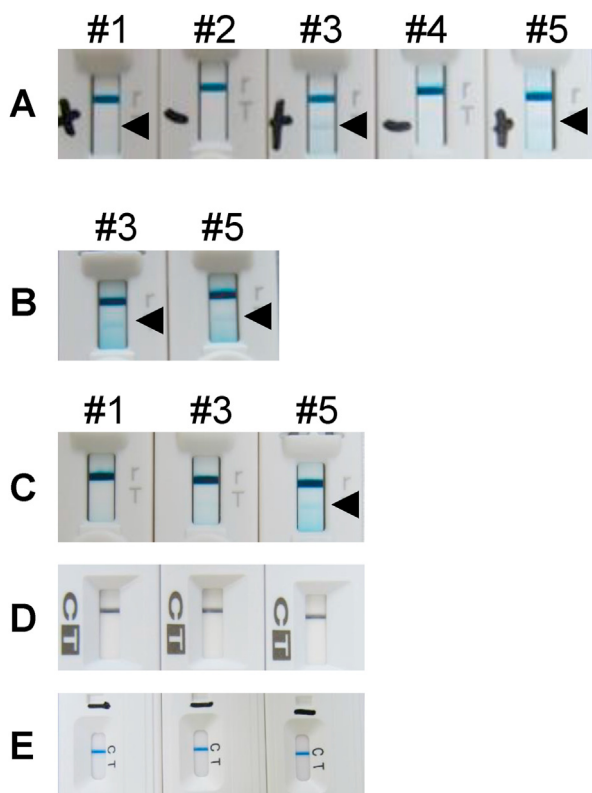
Five patients hospitalized in the ward of disabled persons exhibited a fever for 2 days or more. These patients had been in reverse isolation for the last 7 months since the first wave of COVID-19 in Japan (Table 1). After the onset of fever, tazobactam/piperacillin were empirically administered to all patients, and azithromycin hydrate and favipiravir were additionally administered to the three patients with SARS-CoV-2 RAD test positive. Favipiravir was discontinued after confirming SARS-CoV-2 PCR test negative. Four patients showed defervescence on day 3, but one patient had a persistent fever.

Specimens were collected from the innermost part of the nasal cavity according to the guidelines for the utilization of SARS-CoV-2 antigen detection kits and were prepared for assays according to the respective manufacturer's manual [4].

Three SARS-CoV-2 RAD test kits based on the immunochromatography method are currently available on the Japanese market: ESPLINE® SARS-CoV-2 kit (Lot nos. K4B00811 and K4B00815; Fujirebio Inc., Tokyo, Japan); ImunoAce SARS-CoV-2 kit™ (Lot no. S201003; Tauns Laboratories Inc., Shizuoka, Japan); and

\* Corresponding author. Department of Internal Medicine, National Hospital Organization Awara Hospital, 238-1 Kitagata, Awara, Fukui, 910-4272, Japan.

E-mail address: [kitoth@u-fukui.ac.jp](mailto:kitoth@u-fukui.ac.jp) (K. Itoh).



**Fig. 1.** Detection of SARS-CoV-2 antigen using the ESPLINE SARS-CoV-2 kit (Lot no. K4B00811) on days 1, 2, and 4. The photograph taken on day 1 was taken 12 h after the test was read. Photographs taken on days 2 and 4 were taken immediately after the test was read. #1–#5 indicate patient number. Patients #1–#5 were tested on day 1 (A), and patients #3 and #5 were tested on day 2 (B). Patients #1, #3, and #5 were tested on day 4 using all three SARS-CoV-2 antigen rapid test kits currently available in Japan: ESPLINE SARS-CoV-2 kit (Lot no. K4B00815) (C), ImunoAce SARS-CoV-2 (D), and QuickNavi COVID-19 Ag kit (E). Triangles indicate positive bands.

QuickNavi™ COVID-19 Ag kit (Lot no. 140081; Denka Co., Tokyo, Japan). Each rapid test kit was used according to the respective manufacturer’s manual. At least two laboratory technicians conducted the tests. The SARS-CoV-2 virus genetic test was performed as follows according to the Pathogen Detection Manual [5].

At initial examination (day 1), ESPLINE kit (Lot no. K4B00811) results indicated 3 of 5 patients were positive for SARS-CoV-2. PCR testing was subsequently performed using nasopharyngeal swabs from 57 inpatients in the ward and 12 staff members (e.g., nurses), including the three patients with antigen-positive results.

On day 2, the three patients with antigen-positive results underwent PCR testing using nasopharyngeal swab samples. The RAD test was performed on only two of the three patients because two ESPLINE kits (Lot no. K4B00811) were available. Similar to the first

test results, PCR-negative and antigen-positive results were obtained.

On day 4, antigen tests were performed on the three SARS-CoV-2 antigen-positive patients using ESPLINE (Lot no. K4B00815), ImunoAce, and QuickNavi kits. Of the three false-positive patients detected by the ESPLINE kit (Lot no. K4B00811), one patient with persistent fever was positive by the ESPLINE kit (Lot no. K4B00815). ImunoAce and QuickNavi kits showed negative results. The remaining two patients who had improved fever symptoms were antigen-negative as determined by all three test kits.

We have received a report that rhinoviruses were detected by direct sequencing in the remaining specimens from the PCR test by administrative testing. The rhinoviruses have been confirmed in four patients other than patient no. 4.

### 3. Discussion

The RAD test result using the ESPLINE kit was judged to give false-positive results because patients were PCR-negative, and the occurrence of COVID-19 was clinically denied. We cannot say that there are no true COVID-19 cases in which the antigen test is positive, and the PCR test is negative. However, we estimated that this case was with false positive, taking into account the reported specificity of both the antigen and PCR tests, the fact that there were no PCR test positive cases among the 69 patients and staff involved, and the prevalence situation in the area. In fact, rhinovirus infection was later reported by administrative testing using direct sequencing. Furthermore, because different lots of the ESPLINE kit yielded similar results, and the other two antigen test kits also gave negative results, we speculate that this was an issue specific to the ESPLINE kit.

The RAD test based on the immunochromatography method is widely used in Japan, and if the test is positive, the patient is diagnosed as COVID-19. Although several reports have noted low sensitivity with RAD tests, and false positives have already been reported for quantitative tests such as Lumipulse®, to our knowledge, in qualitative tests no reports have described false-positive results [6–9]. However, more recently, false-positive results from the RAD test have become a problem. The Japanese Association for Infectious Diseases reported that the ESPLINE kit (Lot no. K4B00811) might give false positives, which was the kit used at initial screening [10]. False positives most often occur when a highly viscous sample is used [11]. Although the reported degree of viscosity cannot be accurately defined, the samples were not considered to have high viscosity in this case, and the two antigen-positive cases demonstrated reproducibility as both yielded positive results on the first and second tests. Therefore, these false positives likely did not result from problems with the sample or procedure. There are three types of RAD tests used this time, all of which are based on immunochromatography, but they differ in the coloration principle of the judgment line. ESPLINE uses alkaline

**Table 1**  
Patient characteristics.

Patient no.	Age	Gender	Underlying disease	WBC <sup>a</sup> (/μL)	CRP <sup>a</sup> (mg/dL)	Fever <sup>b</sup>		
						Day 1	Day 2	Day 4
1	<10	M	Down syndrome	4000	2.78	+	+/-	-
2	10	M	Spastic quadriplegia	6900	2.14	+		
3	10	M	Cerebral palsy	18,600	0.82	+	+	+
4	10	M	Cerebral palsy	4300	4.75	+		
5	20	M	Lennox-Gastaut syndrome	4700	14.26	+	+/-	-

CRP, C-reactive protein; M, male; WBC, white blood cell.

<sup>a</sup> Inspection day; Day 1.

<sup>b</sup> Fever; - (<37.0 °C), +/- (37.0–38.0 °C), + (>38.0 °C).

phosphatase labeling for the color reaction, QuickNavi uses colored latex particles for the color reaction, and ImmunoAce uses platinum-gold colloid for the color reaction. We cannot deny the possibility that these different color reactions may have affected the results.

SARS-CoV-2 causes worse outcomes and a higher mortality rate in disabled or older people; thus, the RAD test may play an essential role as primary screening at facilities for the elderly or disabled persons. However, if the RAD test shows positive, the facility and staff will be considerably burdened. In our case, 43 staff members, including nurses, doctors, laboratory technicians, pharmacists, child instructors, and clerks, collectively worked overtime for 147 hours a day. Additionally, our institution experienced some reputational damage. Thus, the use of RAD tests potentially evokes hospital operational risk. It is important to remember that the patient is the one who bears the greatest burden when a false positive occurs. If a patient with false positive result admit to a room for COVID-19, it may increase the risk of nosocomial infection.

We consider that it is desirable to perform PCR testing promptly when patients admitted to the medical treatment ward have symptoms without using a RAD test.

#### Author contribution statement

KI: Data curation, Formal analysis, Investigation, Conceptualization, Methodology, Writing – original draft, review and editing.  
 TK: Data curation, Investigation, Writing – review and editing.  
 YO: Data curation, Investigation, Writing – review and editing.  
 KS: Data curation, Investigation, Writing – review and editing.  
 YS: Data curation, Investigation, Writing – review and editing.  
 CK: Data curation, Investigation, Writing – review and editing.  
 YM: Data curation, Investigation, Writing – review and editing.  
 RH: Data curation, Investigation, Writing – review and editing.  
 HI: Data curation, Investigation, Writing – review and editing.  
 MS: Data curation, Investigation, Writing – review and editing.  
 YM: Data curation, Investigation, Writing – review and editing.  
 KK: Data curation, Investigation, Writing – review and editing.  
 HM: Data curation, Investigation, Writing – review and editing.  
 HT: Data curation, Formal analysis, Investigation, Conceptualization, Methodology, Supervision, Writing – original draft, review and editing.

#### Ethics approval

This study was performed based on the Declaration of Helsinki and its amendments and the Ethical Guidelines for Clinical Research by the Ministry of Health, Labor and Welfare, Japan. Publication of this study was approved by the National Hospital Organization Awara Hospital Clinical Research Review Board (#2024). Since three patients did not have sufficient consent ability,

the test was conducted with the patient's family members' consent, who were the substitutes. If it was possible to communicate easily with the person, the person consented to the inspection.

#### Funding

None.

#### Declaration of competing interest

The authors have read the journal's policy on the disclosure of potential conflicts of interest and the journal's authorship agreement. The authors declare that they have neither conflicts of interest nor competing interests.

#### References

- [1] Andrew M, Searle SD, McElhaney JE, McNeil SA, Clarke B, Rockwood K, et al. COVID-19, frailty and long-term care: implications for policy and practice. *J Infect Dev Ctries* 2020;14:428–32. <https://doi.org/10.3855/jidc.13003>.
- [2] D'Adamo H, Yoshikawa T, Ouslander JG. Coronavirus disease 2019 in geriatrics and long-term care: the ABCDs of COVID-19. *J Am Geriatr Soc* 2020;68:912–7. <https://doi.org/10.1111/jgs.16445>.
- [3] McMichael TM, Currie DW, Clark S, Pogojans S, Kay M, Schwartz NG, et al. Epidemiology of covid-19 in a long-term care facility in king county, Washington. *N Engl J Med* 2020;382. <https://doi.org/10.1056/NEJMoa2005412>. 2005–11.
- [4] Japanese Ministry of Health, Labor and Welfare. The guidelines for the utilization of the SARS-CoV-2 antigen detection kit. Tokyo, Japan: Japanese Ministry of Health, Labor and Welfare; 2020. <https://www.mhlw.go.jp/content/000640554.pdf>. [Accessed 18 November 2020].
- [5] National Institute of Infectious Diseases. The pathogen detection manual 2019-nCoV Ver.2.9.1. Tokyo, Japan: National Institute of Infectious Diseases; 2020. <https://www.niid.go.jp/niid/images/lab-manual/2019-nCoV20200319.pdf>. [Accessed 18 November 2020].
- [6] Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. *J Clin Virol* 2020;129:104500. <https://doi.org/10.1016/j.jcv.2020.104500>.
- [7] Nagura-Ikeda M, Imai K, Tabata S, Miyoshi K, Murahara N, Mizuno T, et al. Clinical evaluation of self-collected saliva by quantitative reverse transcription-PCR (RT-qPCR), direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19. *J Clin Microbiol* 2020;58. <https://doi.org/10.1128/JCM.01438-20>.
- [8] Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as front-line testing for COVID-19 diagnosis. *J Clin Virol: The Official Publication of the Pan American Society for Clinical Virology* 2020;129. <https://doi.org/10.1016/j.jcv.2020.104455>.
- [9] Ogawa T, Fukumori T, Nishihara Y, Sekine T, Okuda N, Nishimura T, et al. Another false-positive problem for a SARS-CoV-2 antigen test in Japan. *J Clin Virol* 2020;131:104612. <https://doi.org/10.1016/j.jcv.2020.104612>.
- [10] The Japanese Association for Infectious Diseases. COVID-19 questionnaire on false positives of the simplified antigen qualitative test (October 27, 2020). Tokyo, Japan: The Japanese Association for Infectious Diseases; 2020. [http://www.kansensho.or.jp/uploads/files/topics/2019ncov/covid19\\_survey\\_201027.pdf](http://www.kansensho.or.jp/uploads/files/topics/2019ncov/covid19_survey_201027.pdf). [Accessed 24 November 2020].
- [11] Fujirebio Inc. Precautions for "ESPLINE SARS-CoV-2" operation and judgment method. [https://www.fujirebio.co.jp/products/espline/pdf/EL\\_SARS-CoV-2\\_ASSAY\\_PROCEDUREFAQ.pdf](https://www.fujirebio.co.jp/products/espline/pdf/EL_SARS-CoV-2_ASSAY_PROCEDUREFAQ.pdf). [Accessed 7 January 2021].