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CASE SERIES

# Adult Influenza Patients Diagnosed by Multiplex Polymerase Chain Reaction Tests, but Not Rapid Antigen Tests, and Managed Successfully During the 2024 to 2025 Major Influenza-Endemic Season in a Tertiary Hospital in Japan

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**Abstract:** We present the two adult cases of influenza diagnosed by multiplex polymerase chain reaction (PCR) in the 2024–2025 season, which was a major season of influenza in Japan. Although rapid antigen tests (RATs) were negative in these two cases, these patients were effectively treated by anti-influenza agents after influenza was definitively diagnosed by multiplex PCR. Relative low sensitivity and specificity might be shown in influenza diagnosis at bedside by RATs, therefore, these data suggest that multiplex PCR could be useful in influenza diagnosis and management during the influenza-endemic season.

**Keywords:** diagnostic accuracy, influenza, multiplex polymerase chain reaction, rapid antigen test, respiratory infections, respiratory syncytial virus, SARS-CoV-2

## Introduction

The influenza virus induces acute febrile illness, malaise and respiratory failure, and influenza is frequently lethal in elderly patients and/or patients with underlying diseases when it develops into pneumonia, encephalopathy, and myocarditis.[1]

In Japan, there have been influenza outbreaks in hospitals and nursing homes in winter, and usually several patients are isolated in private rooms and receive drug therapy with anti-influenza agents, including oseltamivir, laninamivir, and baroxavir.[2,3] In addition, prophylactic administration of anti-influenza agents has been used during outbreaks, and their effectiveness has been proven.[3–5] Therefore, the influenza diagnosis followed by administration of anti-influenza agents not only for treatment, but also for prophylaxis, is critical in the management of patients with influenza and infection control of wards with influenza outbreaks.

In the 2023–2024 season, we saw a very large number of patients with influenza, (18,240,000 patients, of whom 19,389 were hospitalized), although there were only 4,850,000 patients and 3582 hospitalized patients in the 2022–2023 season in Japan as same as the US and the other countries.[2,6,7] Furthermore, there was a rapid increase in the number of influenza patients in the 2024–2025 season (total 10,375,000 patients, and peaked at 2024 in December), and it was not possible to admit severe patients because of an insufficient number of hospital beds although the number of COVID-19 patients were stable.[8]

In these periods, rapid antigen tests (RATs) for influenza have been available, but their sensitivity is not very high, generally around 60%, although genetic diagnostic tests, including polymerase chain reaction tests, such as multiplex PCR tests (FilmArray Respiratory Panels 2.1, bioMerieux, Marcy l'Etoile, France) indicates the positive viral genes when the cycle thresholds were 37 and less, and have shown a very high sensitivity of around 90%.[9,10] Therefore, we

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may need the PCR rather than RATs frequently in the diagnosis for influenza and other respiratory virus infections to start treatment and prophylaxis as early as possible. These recommendation for PCR or start treatment by clinical diagnosis will be critical when the RATs showed negative for influenza although the patients had typical symptoms in the influenza-endemic seasons and areas in the US and the other countries.[3,11]

In this study, multiplex PCR tests were performed for febrile patients who were not diagnosed by RATs for influenza virus (ImmunoAce-Flu, Tauns, Shizuoka, Japan), respiratory syncytial virus (RSV) (Quicknavi-RSV2, Ohtsuka Co. Ltd, Tokushima, Japan), human metapneumovirus (hMPV) (Quickchaser, Mizuho Medy, Saga, Japan), and SARS-CoV-2 (SARS-CoV-2 rapid antigen test, Roche diagnostics, Basel, Switzerland), and some patients who were infected with respiratory viruses other than SARS-CoV-2, such as influenza virus, were identified by nasopharyngeal swabs. Two influenza patients were finally diagnosed by multiplex PCR and received effective treatment with anti-influenza agents. Their conditions improved after appropriate diagnosis followed by definitive influenza treatment.

The samples were collected by cotton swabs within one to two days after symptoms onset, and the RATs and/or multiplex PCR were performed immediately at the clinical laboratory within 30 min after samples collection at the bedside and transport without any unnecessary medium. Patients <18 years were excluded and the samples were collected prospectively.

The review of these cases and the related study were approved by the Institutional Review Board of Saitama Medical University International Medical Center (approval no. 2022–146; approval date: March 1, 2023) and registered as UMIN000047691. The patients whose specimens were used provided written, informed consent for use of their data in the present study. The data have been kept securely in the box with keys. This study adhered to the Declaration of Helsinki.

## Case Series

From October 2024 to March 2025 in which influenza outbreak was actually shown, 30 patients developed fever and respiratory symptoms, including sputum, sore throat, dyspnea and cough, but RATs detected no viruses. In our hospital, the flowchart of febrile patients and/or the patients with respiratory symptoms those could not detect the viral antigens by RATs indicated the moving forward to genetic examinations when the viral infections strongly suspected, therefore, multiplex PCR was performed, and eight patients (8/30; 26.3%) were diagnosed as having influenza (flu), human rhinovirus/enterovirus (hRV/EV), adenovirus (Adeno), parainfluenza 4 (PIV4), and human metapneumovirus (hMPV) (Figure 1). There are no co-infection cases of representative respiratory virus detected by this multiplex PCR system. Of them, influenza was the most detected virus, and two of three patients with influenza could not be diagnosed after two or more RATs for influenza. Therefore, usefulness of PCR could be shown in influenza cases rather than SARS-CoV-2 and the other respiratory virus cases.

These representative two cases are presented below.

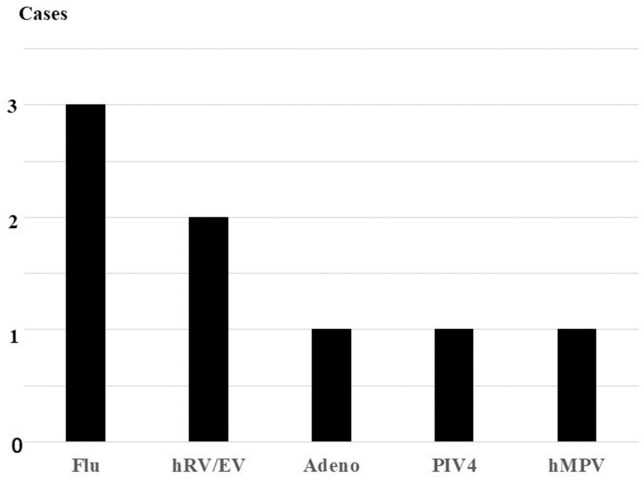


**Figure 1** Viruses detected by multiplex PCR in patients with fever and respiratory symptoms who could not be diagnosed by rapid antigen tests (RATs).
**Abbreviations**: Flu, influenza virus; hRV/EV, human rhinovirus/enterovirus; Adeno, adenovirus; PIV4, parainfluenza virus 4; hMPV, human metapneumovirus.

**Table 1** The Laboratory Data of the Patients 1 and 2

| Items | Data | | Normal Range |
|---|---|---|---|
| | Patient 1 | Patient 2 | |
| White Blood Cells (WBC) | 8.22 × $10^3$/μL | 4.59 × $10^3$/μL | (3.3–8.6) |
| Hemoglobin | 10.4 g/dL | 13.8 g/dL | (13.7–16.8) |
| Platelet | 320.0 × $10^3$/μL | 216.0 × $10^3$/μL | (158.0-348.0) |
| Serum creatinine | 1.25 mg/dL | 0.79 mg/dL | (0.65–1.87) |
| Blood urea nitrogen | 29.9 mg/dL | 9.5 mg/dL | (8–20) |
| Aspartate aminotransferase (AST) | 66 U/L | 91 U/L | (13–39) |
| Alanine aminotransferase (ALT) | 33 U/L | 57 U/L | (10–42) |
| C-reactive protein (CRP) | 3.8 mg/dL | 5.4 mg/dL | (0–0.14) |

## Case/Patient 1

A 66-year-old man who had malignant lymphoma admitted to our hospital with a fever (38.1°) and dyspnea ($SpO_2$ 95%, room air) that had developed 3 days earlier (Day −3). Heatstroke was suspected because RATs for influenza and COVID-19 were negative at admission in summer, but he had had close contact with COVID-19 (Day 0). He had also received ampicillin/sulbactam 3 g three times per day and $O_2$ 1L, but 2 days later (Day 2), he again showed a high fever, although his fever decreased after fluid replacement, and COVID-19 was suspected. The laboratory data at Day 2 is shown in (Table 1; Patient 1). There were no clear infiltration shadows and ground-glass opacities on chest X-ray (Figure 2A), but multiplex PCR showed the genes of influenza virus, but not SARS-CoV-2. He was thus diagnosed with influenza and started on peramivir intravenously for 2 days at 300 mg dosage once daily. He recovered to a non-febrile condition without oxygen 3 days later (Day 5).

## Case/Patient 2

A 68-year-old man who had gastric cancer had been hospitalized and developed fever (38.2°) and respiratory symptoms, such as dyspnea ($SpO_2$ 94%, room air), cough and sputum (Day 0). He was diagnosed with bronchopneumonia and received intravenous antibiotics, including ampicillin/sulbactam 3 g three times per day and levofloxacin, 0.5 g once per day with $O_2$ 1L, but his condition and fever did not improve. The laboratory data were not identical to bacterial pneumonia (Table 1, Patient 2), and his chest X-ray did not show clear infiltration shadows and ground-glass

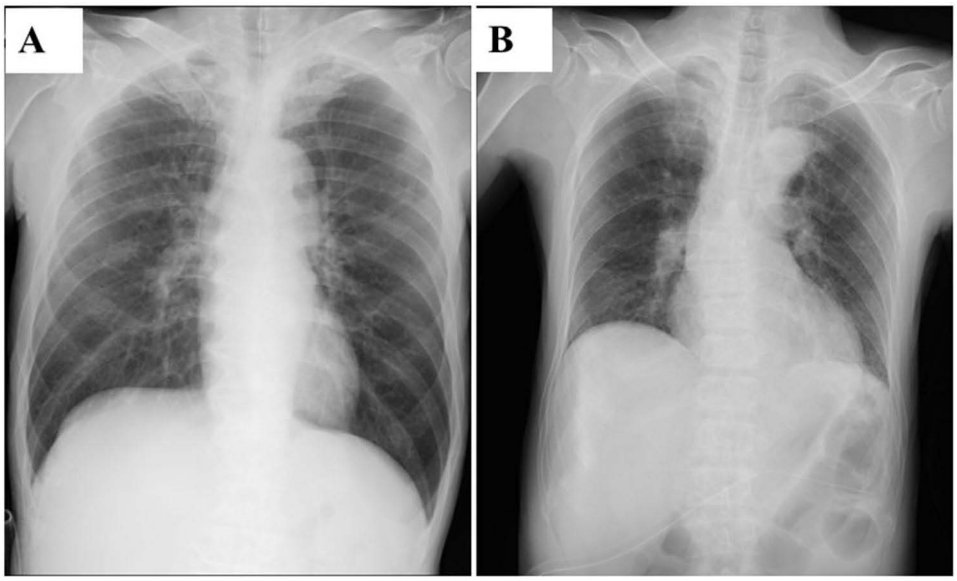


**Figure 2** Chest X-rays on the day that Case 1 (**A**) and Case 2 (**B**) were diagnosed with influenza by multiplex PCR.


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opacities (Figure 2B) at Day 0. Since there was an influenza outbreak on his ward, influenza infection was suspected, and RATs were performed, but the results were influenza-negative despite undergoing three RATs (Days 1 to 3). However, multiplex PCR detected the genes of influenza virus at Day 3, and he was finally diagnosed with influenza and started on laninamivir inhalation 40 mg once, and he recovered to a non-febrile condition without oxygenation 2 days later.

Both of these cases were improved by appropriate influenza therapy after the diagnosis of influenza was made by multiplex PCR.

## Discussion

In this report, 8/30 (26.7%) cases were diagnosed as having viral infections other than SARS-CoV-2 by multiplex PCR, although they could not be diagnosed by RATs. In the same period, 12 SARS-CoV-2 patients were identified by multiplex PCR first, but SARS-CoV-2 antigen was detected in all 12 SARS CoV-2 patients by RATs (data not shown). These data suggest that multiplex PCR might not be needed for the SARS-CoV-2 diagnosis because SARS-CoV-2 viral titers might be high when patients present with fever and respiratory symptoms, unlike patients with influenza.[12–14]

Of the eight cases in whom viral genes were detected by multiplex PCR, but viral antigens were not detected by RATs, influenza virus was the most common, which suggests that multiplex PCR tests may be better than RATs in latent influenza cases.[15,16]

In influenza, RATs might be less sensitive to detect influenza virus than multiplex PCR because influenza virus could induce severe inflammation from the early phase of the infection although the virus titer might be low.[12] Therefore, false-negative rates of RATs were relatively higher in febrile influenza cases than in symptomatic SARS-CoV-2 infection cases, which usually showed viral titers high enough for viral antigens to be detected. Therefore, in Japan, RATs for influenza are recommended at the more than 24 h after onset, rather than very early timing of the influenza onset because the virus may be not enough at the very early stage of the influenza.[5,12,16]

Recently, WHO guideline recommended the PCR-based tests rather than RATs in influenza diagnosis, and we could start treatment for influenza without positive results of RATs because it might be early and showed not only clinical effectiveness but also cost effectiveness.[11] Quality-adjusted life years (QALY) will worsen in the cases with RATs, rather than those with PCRs and/or immediate influenza treatments.[11] RATs might be useful, however, its use will decrease near future, compared with PCR-based tests for influenza diagnosis in Japan as same as US and the other countries.

In addition, multiplex PCR could detect the co-infection of respiratory virus. We could not find any co-infection cases in this study, however, it was reported that influenza and co-infection with the other respiratory virus, including SARS-CoV-2 and RSV are frequent and enhance the severity of the patients' condition.[17] From this view, multiplex PCR has the additional merit that could find the co-infection cases early and prevent the progression to the severe viral pneumonia by additional virus treatments.

As mentioned above, there were so many patients with influenza in the 2023–2024 season.[2] In addition, in the 2024–2025 season, a huge number of influenza patients increased rapidly and we could not accept the admission of severely ill patients, and there appeared to be increased mortality, especially in elderly patients with influenza.[8] Early and appropriate diagnosis followed by early treatment by anti-influenza agents within 48 h is strongly recommended in influenza management in severe, hospitalized patients.[3]

As the limitation, the timeframe of this study from October 2024 to March 2025-appropriately covers a full influenza season, allowing for meaningful observation of diagnostic outcomes. As a case series, the work does not require statistical inference; however, selection bias (since only RAT-negative cases were included) and possible confounding by prior antibiotic use might be considered. The study size was small.

Further study by the huge population design and applications of multiplex PCR rather than RATs will be needed in influenza-endemic seasons for febrile patients with respiratory symptoms not diagnosed by RATs.

## Conclusions

In conclusion, viral genes were detected, and viral infections, including influenza, were identified in febrile patients with respiratory symptoms who could not be diagnosed by RATs. Influenza cases were the most common, and two influenza

patients improved with appropriate administration of anti-influenza agents after definitive diagnosis by multiplex PCR. It might be better to use multiplex PCR rather than RATs especially in influenza management. More detailed and large-scale study will be needed to show the importance of the PCR-based diagnosis at the bedside in influenza management.

## Disclosure

The author reports no conflicts of interest in this work.

## References


1. Mauad T, Hajjar LA, Callegari GD, et al. Lung pathology in fatal novel human influenza A (H1N1) infection. *Am J Respir Crit Care Med*. 2010;181:72–79. doi:10.1164/rccm.200909-1420OC
2. Japan Institute for Health Security. Influenza 2023-2024 season in Japan. *IASR*. 2024;45(11):179–181.
3. Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical practice guidelines by the infectious diseases society of america: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenzaa. *Clinical Infect Dis*. 2019;68:895–902. doi:10.1093/cid/ciy874
4. Nakano T, Shiosakai K. Spread of viral infection to family members from influenza patients treated with a neuraminidase inhibitor. *J Infect Chemother*. 2014;20:401–406. doi:10.1016/j.jiac.2014.01.012
5. Ikematsu H, Hayden FG, Kawaguchi K, et al. Baloxavir marboxil for prophylaxis against influenza in household contacts. *New Engl J Med*. 2020;383:309–320. doi:10.1056/NEJMoa1915341
6. Centers for Diseases Control and Prevention. 2024-2025 Influenza Season News 2025. Available from: https://www.cdc.gov/acip/downloads/slides-2025-06-25-26/03-dugan-influenza-508.pdf. Accessed December 15, 2025.
7. Interim Australian Centre for Disease Control. Australian respiratory surveillance report 2025:1–32.
8. FluNet. 2025: Available from: https://www.who.int/tools/flunet. Accessed December 15, 2025.
9. Geyer RE, Kotnik JH, Lyon V, et al. Diagnostic accuracy of an at-home, rapid self-test for influenza: prospective comparative accuracy study. *JMIR Public Health Surveill*. 2022;8(2):e28268. doi:10.2196/28268
10. Huang HS, Tsai C, Chang J, Hsu TC, Lin S, Lee CC. Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis. *Clin Microbiol Infect*. 2018;24(10):1055–1063. doi:10.1016/j.cmi.2017.11.018
11. World Health Organization. Guidelines for the clinical management of severe illness from influenza virus infections WHO website 2022. Available from. Accessed December 15, 2025.
12. Dee K, Schultz V, Haney J, Bissett LA, Magill C, Murcia PR. Influenza A and respiratory syncytial virus trigger a cellular response that blocks severe acute respiratory syndrome virus 2 infection in the respiratory tract. *J Infect Dis*. 2023;227(12):1396–1406. doi:10.1093/infdis/jiac494
13. Chaimayo C, Kaewnaphan B, Tanlieng N, et al. Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. *Virol J*. 2020;17(1):177. doi:10.1186/s12985-020-01452-5
14. Nordgren J, Sharma S, Olsson H, et al. SARS-CoV-2 rapid antigen test: high sensitivity to detect infectious virus. *J Clin Virol*. 2021;140:104846. doi:10.1016/j.jcv.2021.104846
15. Su X, Zho J, Liu L, et al. Performance evaluation of influenza a rapid antigen test and PCR among nasopharyngeal and oropharyngeal samples. *Pract Lab Med*. 2024;40:e00416. doi:10.1016/j.plabm.2024.e00416
16. Tsushima Y, Uno N, Sasaki D, Morinaga Y, Hasegawa H, Yanagihara K. Quantitative RT-PCR evaluation of a rapid influenza antigen test for efficient diagnosis of influenza virus infection. *J Virol Methods*. 2015;212:76–79. doi:10.1016/j.jviromet.2014.10.019
17. Swets MC, CD R, EM H, et al. SARS-CoV-2 co-infection with influenza viruses, respiratory syncytial virus, or adenoviruses. *Lancet*. 2022;399(10334):1463–1464. doi:10.1016/S0140-6736(22)00383-X