Title:

Multicenter evaluation of molecular point-of-care testing and digital immunoassays for influenza virus A/B and respiratory syncytial virus in patients with influenza-like illness

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Authorship statement:

All authors meet the ICMJE authorship criteria. All authors developed the trial designs and were responsible for the organization and coordination of the trial. Kaku N, Akamatsu N, Kodama H, and Yanagihara K were responsible for the data analysis. Kaku N, Narita Y, Matsuoto Y, Matsushita T, Mizuta Y, and Yanagihara K contributed to the management or administration of trial. All authors contributed to the writing of the final manuscript.

Abstract:

Introduction: Digital immunoassays (DIAs) and molecular point-of-care (POC) tests for influenza were recently developed. We aimed to evaluate and compare the positive rate with molecular POC tests and DIAs in detecting influenza virus A, B and respiratory syncytial virus (RSV).

Methods: A prospective observational study was conducted in 2019–2020. Nasopharyngeal swab samples were collected from adult outpatients with influenza-like illness who visited four hospitals and clinics in Japan. DIAs were performed at each facility. The clinical diagnosis was determined based on the findings of DIAs, history taking, and physical assessment. Molecular POC test and reverse transcription polymerase chain reaction (RT-PCR) were performed later.

Results: A total of 182 patients were evaluated. The positive rate for influenza virus with molecular POC test was significantly higher than that with DIAs (51.6% versus 40.7%, p = 0.046). In patients who tested positive for influenza virus with only molecular POC test, the presence of influenza virus was confirmed by RT-PCR. In a comparison between the patients who were positive for influenza virus with only molecular POC test and those with both molecular POC test and DIA, the percentage of patients who sought consultation within 18 hours after the onset of symptoms was significantly higher in the molecular POC test only group than in the both methods group (70.0% versus 43.2%, p = 0.044).

Conclusions: A molecular POC test could contribute to the accurate diagnosis of influenza in patients with influenza-like illness, especially those who visited a hospital immediately after the onset of symptoms.

Keywords: influenza; molecular diagnostic techniques; multicenter study; point-of-care; respiratory syncytial virus

Introduction:

Influenza is an acute respiratory illness caused by influenza virus A or B that occurs epidemic. Additionally, influenza virus is one of the few respiratory tract viruses that can be treated with antiviral agents. However, influenza is difficult to diagnose, because its symptoms are similar to those of other common illnesses.[1] Accordingly, immunocompromised patients and those at higher risk for developing influenza complications with influenza-like illness should undergo diagnostic tests for detection of the influenza virus. This can help avoid unnecessary laboratory tests to detect other etiologies and the inappropriate use of antibiotics, improve the effectiveness of infection prevention and control measures, and increase the use of appropriate antiviral agents.[2] Influenza antigen tests have been used worldwide for the diagnosis of influenza. However, a meta-analysis of observational studies revealed that it had low sensitivity (62%) in the detection of influenza virus.[3] Thus, physicians often suspect a false-negative result and clinically diagnose patients with influenza when the result of the antigen test is negative.

Recently, automated immunochromatographic antigen tests (digital immunoassays [DIAs]) and molecular point-of-care (POC) tests for influenza have been developed that have become popular mainly in Europe and North America.[4] In this study, we aimed to evaluate the positive rate with molecular POC tests using the cobas Liat system (Liat Flu/RSV) in detecting influenza virus A, B and respiratory syncytial virus (RSV) within 20 minutes,[5] DIAs for influenza virus A and B, and DIAs for RSV using nasopharyngeal swab samples obtained from patients from multiple hospitals in Japan. We also compared the backgrounds of the patients who underwent DIA and molecular POC tests to determine the group wherein a molecular POC test could be considered more useful.

Material and methods:

Ethics

This study was approved by the ethics committee of Nagasaki University Hospital (approval number: 19102103) and was registered at the UMIN Clinical Trials Registry (reference number: UMIN000037969). Written informed consent for participation in and publication of this study was obtained from all participants before sample collection.

Study design

A prospective observational study was conducted between November 15, 2019 and March 14, 2020 in Nagasaki University Hospital, Narita Naika Clinic, Matsumoto Naika, Shinzato Medicare Group Shinzato Clinic, and Menoto Hospital. Adult outpatients with one or more symptoms, such as fever (≥38°C or an increase of ≥1°C from normal body temperature), nasal discharge, nasal congestion, sore throat, cough, headache, chills, fatigue, joint pain, or muscle pain, who visited the Narita Naika Clinic, Matsumoto Naika, Shinzato Medicare Group Shinzato Clinic, and Menoto Hospital were included in this study. Patients treated with anti-influenza agents 1 month prior to the clinic or hospital visit were excluded. After obtaining informed consent, two sets of nasopharyngeal swab samples were collected: one was used in the DIAs for detection of influenza virus and RSV, and the other was stored in 3 mL of UTM (Copan Italia spa, Brescia, Italy) at −80°C until further analysis using molecular POC test and reverse transcription polymerase chain reaction (RT-PCR). The physicians determined the clinical diagnosis on the basis of history taking, physical assessment, and DIAs, from which they produced a clinical report for each patient. Molecular POC test using Liat Flu/RSV (Roche Molecular Systems Inc, NJ, USA) was performed at Nagasaki University Hospital. The samples were then transferred to Roche Diagnostics K.K. (Tokyo, Japan) for RT-PCR analysis as the results of DIAs and molecular POC tests varied. Clinical

report forms and results of RT-PCR were summarized and analyzed at Nagasaki University Hospital.

Data collection

Data on patients' characteristics were collected: sex, age, underlying diseases, history of influenza vaccination, time from the onset of symptoms, maximum body temperature before consultation, body temperature at the time of consultation, clinical diagnosis, underlying diseases, current medication, results of antigen test, and signs and symptoms (fever [≥38°C or an increase of ≥1°C from normal body temperature] cough, sore throat, nasal discharge, headache, arthralgia, myalgia, fatigue, nausea, and diarrhea).

DIAs and molecular POC test

DIAs for detection of influenza virus (BD Veritor System Flu, Becton, Dickinson and Company, Tokyo, Japan) and RSV (BD Veritor System RSV, Becton, Dickinson and Company, Tokyo, Japan) were performed in each facility according to the manufacturer's instructions. Molecular POC test was performed in Nagasaki University Hospital according to the manufacturer's instructions. RT-PCR for influenza virus or RSV was performed in Roche Diagnostics K.K. using samples from patients with different DIA and molecular POC test results. RNA was extracted from samples using the QIAamp Viral RNA Mini Kit (QIAGEN N.V., Hilden, Germany); next, real-time RT-PCR was performed using LightCycler 480 Instrument II (Roche Diagnostics Ltd., Inc, Rotkreuz, Switzerland) and RealStar® Influenza RT-PCR Kit 2.0 or RealStar® RSV RT-PCR Kit 3.0 (Altona Diagnostics GmbH, Hamburg, Germany) according to the manufacturers' instructions. A crossing point value was calculated using the second derivative maximum method.

Statistical analysis

All statistical analyses were performed using EZR version 1.53 (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[6] which is a graphical user interface for R (the R Foundation for Statistical Computing, Vienna, Austria; version 4.0.3). Fisher's exact test was used to compare categorical variables. Continuous variables were expressed as mean ± standard deviation and compared using the Student's t-test. The statistical significance level was set at <0.05.

Data availability

Raw data were obtained from Nagasaki University Hospital and Roche Diagnostics K.K.. Derived data supporting the findings of this study are available from the corresponding author on request.

Results:

Patients' characteristics

During the study period, a total of 182 patients were evaluated. The patients' characteristics are shown in Table 1 and Figure 1. The average age of the patients was 47.9 ± 17.7 years, and 33.0% of the patients had underlying diseases. Of the total patients, 70 (38.5%) and 124 (68.1%) visited a hospital or clinic within 12 h and 24 h, respectively, after the onset of symptoms (Figure 1). The common symptoms reported were fever (93.4%), fatigue (83.5%), cough (71.4%), sore throat (62.6%), and arthralgia (61.5%). Of the total patients, 87 (47.8%) were clinically diagnosed with influenza, and 85 (46.7%) were administered anti-influenza agents. In addition, 2 (1.1%) patients were clinically diagnosed with RSV infection.

Comparison of DIAs and molecular POC test

The results of DIAs and molecular POC test are shown in Table 2. The positive rate for influenza virus with molecular POC test was significantly higher than that with DIAs (51.6% versus 40.7%, $p = 0.046$). Twenty patients showed different results with DIAs and molecular POC test for the influenza virus (Figure 2A), and in all patients, molecular POC test showed a positive result, whereas DIAs yielded a negative result. The presence of influenza virus A was confirmed in all 20 patients by conducting an RT-PCR analysis.

The positive rate for RSV with molecular POC test was the same as that with DIAs, but two patients showed different results (Figure 2B). One patient tested positive for RSV in addition to influenza virus in molecular POC test, but tested negative for RSV in DIAs. The presence of RSV was confirmed in a sample obtained from this patient by RT-PCR. The other patient tested negative for RSV with molecular POC test, but positive with DIAs. RSV was not detected in a sample obtained from this patient by RT-PCR.

Comparison of patients with different results from diagnostic tests for influenza virus

We compared the backgrounds of 20 patients who tested positive for influenza virus with the molecular POC test only (molecular test only group) and 74 patients who tested positive for influenza virus with both molecular POC test and DIAs (both methods group) to determine the study group wherein a molecular POC test could be more useful than DIAs. With respect to backgrounds, the percentage of patients who visited clinics or hospitals within 18 hours after the onset of symptoms was significantly higher in the molecular test only group than in the both methods group (70.0% versus 43.2%, $p = 0.044$). By contrast, the percentage of patients who visited clinics or hospitals 24 hours after the onset of symptoms was significantly lower in the molecular test only group than in the both methods group (10.0% versus 39.2%, $p = 0.016$). Although the prevalence of influenza vaccination and nasal

discharge was lower in the molecular test only group than in the both methods group, no significant difference was observed ($p = 0.065$ and 0.075, respectively).

In this study, the physicians determined the clinical diagnosis on the basis of history taking, physical assessment, and antigen tests. In the molecular test only group, 7 patients (35.0%) were diagnosed with influenza, but the rest were diagnosed with other diseases. The percentage of patients treated with anti-influenza agents was significantly lower in the molecular test only group than in the both methods group (40.0% versus 98.6%, $p < 0.001$). The percentage of patients treated with antibiotics was significantly higher in the molecular test only group than in the both methods group (25.0% versus 1.4%, $p = 0.001$).

Discussion:

In this study, the positive rate for influenza virus was significantly higher with molecular POC test than with DIAs. The presence of influenza virus was confirmed by RT-PCR in 20 patients who tested positive for influenza virus with molecular POC test, but negative with DIAs. The sensitivity of DIAs for influenza virus was 0.787, if true positive for influenza virus was defined as a positive result from either molecular POC test or RT-PCR. A previous study that compared the Liat Flu/RSV with traditional antigen test for detection of influenza virus in Japan reported a sensitivity of 0.571 for the traditional antigen test, if true positive for influenza virus was defined as a positive result from either molecular POC test or RT-PCR. A previous meta-analysis that compared the traditional antigen tests, DIAs, and molecular POC tests for influenza reported that the pooled sensitivity of these tests for influenza virus A was 0.544, 0.800, and 0.916, respectively.[4] These results, including those from our study, indicated that DIAs had an improved sensitivity for influenza virus than the traditional antigen tests, but had less sensitivity than molecular POC test. In this study, physicians clinically diagnosed 7 patients (35.0%) with influenza, and 8 (40.0%) were treated with anti-

influenza agents, even if they tested negative for influenza virus with DIAs. Hence, a more accurate diagnosis of influenza is warranted owing to the possibility of co-circulation of SARS-CoV-2 and influenza viruses and difficulties in differentiating between these viruses based on clinical symptoms.[7] Thus, molecular POC test must be performed for the detection of influenza virus.

Although molecular POC test is the best method for diagnosing influenza, the cost of this test is 2–5 times higher than that of DIAs.[4] On the other hand, a positive result from molecular POC test significantly reduced the use of antibiotics and median hospitalization.[8] In this study, 25.0% of the patients with false-negative results in DIAs were treated with antibiotics; antibiotic use could have been reduced if molecular POC test was performed at the time of consultation. In addtion, molecular POC test has been stated as a cost-effective strategy.[9] However, its cost should be considered from a patient's perspective. Therefore, a different diagnostic method may be used depending on the individual situation. We compared the background of patients with different test results to determine the group wherein a molecular POC test could be considered more useful. The percentage of patients who visited a hospital within 18 hours after the onset of symptoms was higher in the molecular POC test only group than in the both methods group. When we analyzed the patients who visited a hospital within 18 hours after the onset of symptoms, the sensitivity of DIAs for influenza virus was 0.696 (32/46). When we analyzed the patients who visited a hospital 24 hours after the onset of symptoms, the sensitivity of DIAs for detection of influenza virus was 0.935 (29/31). These results are similar to those reported in a previous meta-analysis, which evaluated the accuracy of traditional antigen test for influenza virus.[3] Accordingly, molecular POC test should be used instead of DIAs in patients who visited a hospital immediately after the onset of symptoms. Based on the results of this study, the use of molecular POC test can

contribute to the reduction of antibiotic use and increase in the administration of anti-influenza agents in these patients.

We also evaluated the rate of RSV positivity in this study. The positivity rate of both DIAs and molecular POC test for RSV was extremely low (1.1%). RSV causes seasonal outbreaks throughout the world, and outbreaks usually peak in January or February in the northern hemisphere.[10] RSV is the common cause of lower respiratory tract infection in children aged below 1 year.[11] The positivity rate for RSV was extremely low because only adult outpatients participated in this study. In our previous study on multiplex molecular test conducted in adult outpatients during the influenza epidemic, RSV was detected in 12% of these patients.[12] Although no data were available on RSV infection in Japan during the period of our investigation, a previous study on seasonal influenza activity in Japan reported that influenza activity was significantly lower from weeks 3 through 7 in 2019–2020 than in 2014–2015 to 2018–2019.[13] Therefore, the status of the RSV epidemic in this study was possibly different than that in our previous study. Only 1 patient in this study showed matching results with DIAs and molecular POC test for the detection of RSV. Meanwhile, one patient showed a false-negative result with DIAs for RSV based on the results of RT-PCR. A previous systematic review on antigen test for RSV revealed that the sensitivity of antigen test was low in adult patients,[14] and it may have contributed to the false-negative result in DIAs in this study. In addition, one study patient showed a false-positive result in DIAs. This may be due to the prevalence of RSV infection in this study, because the specificity of antigen tests for RSV was reported to be high in adult patients.[14]

This study had some limitations. First, RT-PCR was only performed in samples with different results with DIAs and molecular POC test. Although several studies have reported an extremely low prevalence, the Liat Flu/RSV assay can yield a false-negative or false-positive result[15]; hence, there is a possibility that there were false-negative or false-positive

results in this study. Second, the positive rate for influenza and the rate of the patients who visited a hospital 24 hours after the onset of symptoms seemed higher in this study. Since this study was a single-arm study, there is a possibility that there was a selection bias. Third, only one molecular POC test was evaluated in this study. There are several molecular POC tests that can detect influenza virus, such as GeneXpert Xpress FLU/RSV[16] and ID NOW Influenza A and B assay[17]. Hence, further investigation on the comparison of these molecular POC tests is warranted. Forth, investigations could not be conducted during the RSV epidemic. Although some of the differences between DIAs and molecular POC test for RSV were presented in this study, the exact difference between these two tests could not be accurately determined. Thus, continued investigation is needed to determine the best method for detecting RSV.

Conclusions:

Molecular POC test could contribute to the accurate diagnosis of influenza in patients with influenza-like illness, compared with DIAs. Molecular POC test is useful for patients who visited a hospital immediately after the onset of symptoms.

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Disclosure statement:

This study was funded by Roche Diagnostics K.K. The sponsor also performed RT-PCR analysis in this study. The sponsor had no control over the interpretation, writing, or

publication of this work. The authors have no conflict of interest directly relevant to the content of this article.

List of abbreviations

DIAs: Digital immunoassays

POC: point-of-care

RSV: respiratory syncytial virus

RT-PCR: reverse transcription polymerase chain reaction

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Figures:

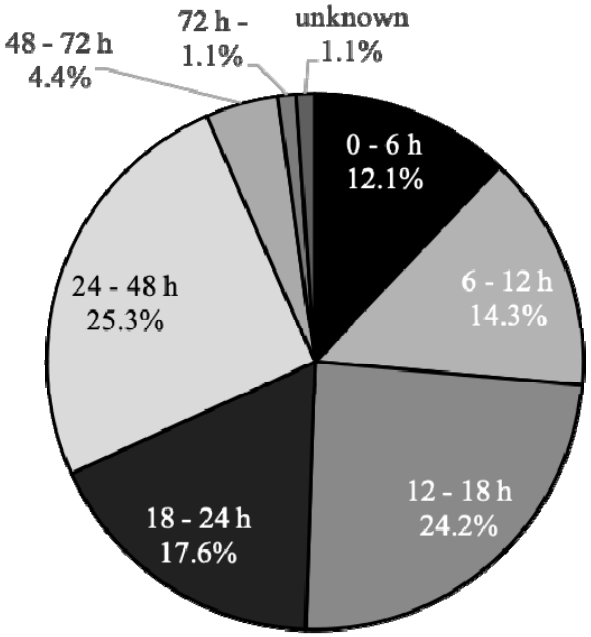

Figure 1. Time of symptom onset
Data on the time of symptom onset were collected when the patients visited the clinics or hospitals.

a)

Influenza		Molecular POC test		
		Pos	Neg	
DIAs	Pos	74	0	74
	Neg	20*	88	108
		94	88	182

b)

RS virus		Molecular POC test		
		Pos	Neg	
DIAs	Pos	1	1†	2
	Neg	1‡	179	182
		2	180	182

Figure 2. Comparison of the results of DIAs and molecular POC test
Influenza (a) and RSV (b) were detected by DIAs and molecular POC test.
*Influenza was detected in all samples by real-time RT-PCR. †RSV was not detected by real-time RT-PCR. ‡RSV was detected by real-time RT-PCR.
Pos, positive for virus; Neg, negative for virus; DIAs, digital immunoassays (automated immunochromatographic antigen tests); POC, point-or-care; RSV, respiratory syncytial virus

Tables:

Table 1. Patients' characteristics

Characteristic	Overall (N=188)	
	N	(%)
Age*	47.9	± 17.7
Sex = female	89	(48.9%)
Underlying diseases	60	(33.0%)
Influenza vaccination	75	(41.2%)
Symptoms		
Fever	170	(93.4%)
Maximum body temperature before consultation*	38.5	± 0.8
Body temperature at the time of consultation*	37.9	± 0.9
Fatigue	152	(83.5%)
Cough	130	(71.4%)
Headache	121	(66.5%)
Sore throat	114	(62.6%)
Arthralgia	112	(61.5%)
Nasal discharge	102	(56.0%)
Myalgia	88	(48.4%)
Nausea	9	(4.9%)
Diarrhea	7	(3.8%)
Clinical diagnosis		
Influenza	87	(47.8%)

RSV infection	2	(1.1%)
Acute upper respiratory infection	63	(34.6%)
Acute bronchitis	19	(10.4%)
Pneumonia	3	(1.6%)
Others	8	(4.4%)
Administration of anti-influenza agents	85	(46.7%)
Administration of antibiotics	36	(19.8%)

*Data are expressed as average ± standard deviation. Fever was defined as follows: ≥38°C or an increase of ≥1°C from normal body temperature.

Table 2. Results of DIAs and molecular POC test

Item	N=182	
	N	(%)
DIAs for influenza virus		7
Influenza virus A	74	(40.7%)
Influenza virus B	0	(0%)
Negative	110	(60.4%)
DIAs for RSV		
Positive	2	(1.1%)
Negative	180	(98.9%)
Molecular POC test		
Influenza virus A*	94	(51.6%)
Influenza virus B	0	(0%)
RSV*	2	(1.1%)
Negative	87	(47.8%)

*One patient was positive for both influenza A and RSV.

DIAs, digital immunoassays (automated immunochromatographic antigen tests); POC, point-or-care; RSV, respiratory syncytial virus

Table 3. Comparison of background of the patients with different results from diagnostic tests for influenza virus

Background	Positive for influenza with molecular POC test only		Positive for influenza with both DIAs and molecular POC test		*p* value
	N = 20	(%)	N = 74	(%)	
Age*	44.3	± 12.8	46.3	± 14.6	NS
Sex = female	9	(45.0%)	37	(50.0%)	NS
Underlying diseases	5	(25.0%)	21	(28.4%)	NS
Influenza vaccination	1	(5.0%)	18	(24.3%)	0.065
Time since onset of symptoms					
0–6 h	4	(20.0%)	7	(9.5%)	NS
0–12 h	6	(30.0%)	16	(21.6%)	NS
0–18 h	14	(70.0%)	32	(43.2%)	0.044
0–24 h	17	(85.0%	47	(63.5%	0.103

		)		)	
24 h	2	(10.0%	29	(39.2%	0.016
		)		)	
Unknown	1	(5.0%)	0	(0.0%)	NS
Symptoms					
Fever	20	(100%)	70	(94.6%	NS
				)	
Maximum body temperature before consultation*	38.5	± 0.7	38.7	± 0.8	
Body temperature at the time of consultation*	38.2	± 0.8	37.9	± 0.9	
Fatigue	16	(80.0%	65	(87.8%	NS
		)		)	
Cough	17	(85.0%	62	(83.8%	NS
		)		)	
Headache	14	(70.0%	51	(63.5%	NS
		)		)	
Sore throat	15	(75.0%	47	(63.5%	NS
		)		)	
Arthralgia	15	(75.0%	49	(66.2%	NS
		)		)	
Nasal discharge	8	(40.0%	47	(63.5%	0.075
		)		)	
Myalgia	11	(55.0%	38	(51.4%	NS
		)		)	

Nausea	0	(0.0%)	2	(2.7%)	NS
Diarrhea	0	(0.0%)	2	(2.7%)	NS
Clinical diagnosis					
Influenza	7	(35.0%)	74	(100%)	<0.001
Acute upper respiratory infection	9	(45.0%)	0	(0.0%)	<0.001
Acute bronchitis	4	(20.0%)	0	(0.0%)	0.002
Administration of anti-influenza agents	8	(40.0%)	73	(98.6%)	<0.001
Administration of antibiotics	5	(25.0%)	1	(1.4%)	0.001

*Data are expressed as average ± standard deviation. Fever was defined as follows: ≥38°C or an increase of ≥1°C from normal body temperature. NS, *p* value was over 0.20.

DIAs, digital immunoassays (automated immunochromatographic antigen tests); POC, point-or-care

Figure legends:

Figure 1. Time of symptom onset

Data on the time of symptom onset were collected when the patients visited the clinics or hospitals.

Figure 2. Comparison of the results of DIAs and molecular POC test

Influenza (a) and RSV (b) were detected by DIAs and molecular POC test.

*Influenza was detected in all samples by real-time RT-PCR. †RSV was not detected by real-time RT-PCR. ‡RSV was detected by real-time RT-PCR.

Pos, positive for virus; Neg, negative for virus; DIAs, digital immunoassays (automated immunochromatographic antigen tests); POC, point-or-care; RSV, respiratory syncytial virus