Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT) by immunochromatography: a cross-sectional study.

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Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT) by immunochromatography: a cross-sectional study.

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Contribution statement

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ABSTRUCT

[Objective] Rapid influenza antigen detection tests (RIADTs) using immunochromatography are widely used among primary care physicians for the diagnosis and management of influenza. However, their low sensitivity has been noted, and some reported factors affecting the results remain controversial. To evaluate the diagnostic accuracy and characteristics of the RIADT and determine which symptoms are relevant to the results.

[Design] A single-center, cross-sectional study.

[Setting] Primary-care centre, Tokyo, Japan.

[Participants] 82 outpatients presenting with flu-like symptoms between December 2010 and April 2011.

[Main Outcome Measures] All patients were physically examined and tested by both the RIADT and a fully automated respiratory virus nucleic acid test (Verigene Respiratory Virus Plus; RV+), the latter being the "gold standard." Based on test results, patients were divided into 4 groups: False Negative (FN), RIADT- and GS+; True Positive (TP), RIADT+ and GS+; True Negative (TN), RIADT- and GS-; and False Positive (FP), RIADT+ and GS-. Groups were then compared regarding age, sex, body temperature, timing of the test from symptom onset, vaccination record and symptoms (sore throat and/or pharyngeal erythema, arthralgia and/or myalgia, headache, chills, cough and/or sputum, and nasal discharge).

[Key Results] The sensitivity, specificity, positive predictive value and negative predictive value of the RIADT were 72.9%, 91.3%, 95.6% and 56.8%, respectively. When the FN and TP groups were compared, the time from symptom onset to the test was shorter the FN group (p=0.0010). No significant differences were detected for the other factors assessed. Comparison of the FN and TN groups revealed that more FN patients had chills than did TN patients (p=0.032).

[Conclusions] Consistent with previous reports, the sensitivity of the RIADT used was low,

probably due to early administration of the test. In patients with negative RIADT results, presence of chills might predict influenza.

ARTICLE SUMMARY

Article focus

- Influenza is one of the most common diseases that general physicians see clinically. The RIADT is helpful for diagnosis, but its low sensitivity is sometimes misleading, resulting in underdiagnosis.
- We aimed to evaluate the diagnostic accuracy and characteristics of the RIADT and determine which symptoms are relevant to the results.

Key messages

- Clinically, the sensitivity of RIADT is relatively low, as was our result.
- Testing too early is suggested to be a factor increasing false negative result.
- The presence of chills is supposed to be an indicator of influenza if the RIADT result is negative.

Strengths and limitations of this study

- Our findings may help general physicians when using the RIADT.

The following limitations do need to be acknowledged. First, the 82 subjects enrolled in the current study were relatively young and healthy. Second, the reference or "gold" standard in this study was not viral culture or RT-PCR but analysis using the Verigene System RV+, which detects influenza viral nucleic acid.

INTRODUCTION

Influenza is a rapid-onset systemic illness caused by influenza virus; patients present with high fever, chills, cough, myalgia, sore throat and headache.[1, 2] Previously, the diagnosis was made from these symptoms.[3-6] However, since their introduction to Japan in 1999, rapid influenza antigen detection tests (RIADTs) using immunochromatography have dramatically changed the influenza diagnostic procedure.[7,8] Before the introduction of RIADTs and anti-influenza drugs, physicians told patients to stay home if they had no suspected complications. Now physicians use RIADTs to diagnose flu and therefore can prescribe anti-influenza viral drugs soon after symptom onset.[8-13] Making the distinction between flu and other respiratory diseases is valuable, and serves to improve individual care management.[14-16] Detection of influenza virus can reduce inappropriate antibiotic use, guide antiviral therapy, and decrease use of other laboratory studies and health care costs.[14] Currently, many RIADTs are available, the sensitivities and specificities have been improved and their usefulness has been widely recognized.[17] RIADTs help physicians diagnose influenza during epidemics, but the RIADT results make it difficult to diagnose flu during periods of transition from epidemic to non-epidemic times, or when patients present with atypical symptoms.[17,18]

For example, when patients' flu-like symptoms are typical of influenza but the RIADT results are negative, whether the patient actually has influenza is unclear. Physicians may question whether samples were obtained correctly or whether the result is a false-negative, and may hesitate to prescribe anti-influenza drugs.

According to a meta-analysis reported in 2012, the pooled RIADT sensitivity was 62.3% (95% CI, 57.9-66.6%), and specificity was 98.2% (95% CI, 97.5-98.7%).[19] Thus, the

specificity is very high but the sensitivity is relatively low. Several factors affecting the results of RIADTs have been reported, but some remain controversial.[19-20]

This study was designed to evaluate the diagnostic accuracy and characteristics of one RIADT, the RapidTesta FLUII (Sekisui Medical, Tokyo, Japan), and to determine which symptoms are associated with the results obtained. In addition, we sought to identify predictors of influenza for patients with false negative RIADT results.

METHODS

From December 2010 to April 2011, a total of 82 outpatients presenting with flu-like symptoms to the Departments of General Medicine, Juntendo University Hospital and Juntendo University Nerima Hospital, both in Tokyo, Japan, were assessed. All of the patients were physically examined and tested by both the RIADT and a fully automated respiratory virus nucleic acid test, Verigene Respiratory Virus Plus; RV+ (NanoSphere, Chicago, IL, USA), the latter being the "gold standard" (GS). Each patient's age, sex, body temperature, time from symptom onset, vaccination record and symptoms (sore throat and/or pharyngeal erythema, arthralgia and/or myalgia, headache, chills, cough and/or sputum, and nasal discharge) were checked and recorded for subsequent analysis to find relationships with the results of the RIADT. Based on the test results, patients were divided into 4 groups, as follows: False Negative (FN), RIADT- and GS+; True Positive (TP), RIADT+ and GS+; True Negative (TN), RIADT- and GS-; and False Positive (FP), RIADT+ and GS-. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RIADT were calculated. In order to ascertain the cause of false-negative results and to find a predictor of influenza, comparisons were made between pairs of groups (FN vs. TP and then FN vs. TN) with respect to age, sex,

vaccination, time from symptom onset to test, body temperature and symptoms.

Laboratory Confirmation

Two nasopharyngeal specimens were collected from each patient by a physician, using sterile cotton swabs, following the procedure detailed in the RIADT manufacturer's package insert.

1. RIADT

The RapidTesta FLU II was performed using one of the collected specimens, according to the manufacturer's instructions. In brief, samples were diluted in the medium immediately, and then dripped into the test device. Results were determined 15 minutes after the test start.

2. Fully automated respiratory virus nucleic acid test

The second swab was immediately placed into Universal Viral Transport medium (UTM; Becton Dickinson, Tokyo, Japan) and tested by the Verigene System within 24 hours. The system uses a multiplexed microarray-based technology. A nucleic acid detection cartridge named Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) was selected, which could detect the following influenza viruses: A (H1N1), A (H3N2), pandemic 2009 influenza A (H1N1), influenza B virus, and respiratory syncytial virus (A, B). This system was approved by the American Food and Drug Administration (FDA) in May 2009.[21]

Specimens were tested with the RV+ system according to the manufacturer's instructions by the physician attending the study patients. Briefly, the test cartridge was pre-loaded with wash solutions, oligonucleotide probe solution and signal amplification solution. The RV+ extraction

tray, amplification tray and test cartridge were then loaded onto the Verigene System. Following the addition of 200 microliters of UTM containing material expressed from the nasopharyngeal swabs, the analysis, which consisted of a programmed, totally automated extraction, RT-PCR, and hybridization sequence, started. The final readout of the microarray was made by the insertion of the slide array into a reader.[22]

Statistical Analysis

The sensitivity, specificity, PPV and NPV of the RIADT used were determined using standard methods. Statistical analysis was performed using a data analysis and statistical software package, STATA SE 12 (StataCorp LP, College Station, Texas, USA). Continuous variables (age, the time from symptom onset, and body temperature) were analyzed by Student's t test, and the chi-squared test was used for comparing patient sex and symptoms. Significance was assigned to results having P values <0.05.

Ethical Statement

This study was reviewed and approved by the internal review boards of Juntendo University Hospital (approval number 22-290) and Juntendo Nerima Hospital (approval number 10-26).

RESULTS

A total of 82 patients (Juntendo University Hospital: 37 patients; Juntendo University Nerima Hospital: 45 patients) were assessed from January 2010 to April 2011. Table 1 shows the characteristics for all patients. The median age was 30.5 (range 20-63) years, and 42.7% (35/82)

were men. During the 2010/2011 flu season, 48.8% (40/82) were vaccinated for influenza. The average time from symptom onset to diagnostic test was 18.9±17.2 hours. 13.4% (11/82) came to hospital within 6 hours from symptom onset, and 72.0% (59/82) within 24 hours.

Table 2 shows the results of RIADT and RV+ as well as the accuracy of RIADT. By RIADT results, 54.9% (45/82) patients were positive and the other 45.1% (37/82) were negative. By RV+ results, 72.0% (59/82) were positive and the other 28.0% (23/82) were negative. Dividing them into 4 groups, the numbers in each were: FN, 16; TP, 43; TN, 21; and FP, 2. The prevalence of influenza A or B virus infection was 72.0%. The sensitivity, specificity, PPV and NPV were 72.9%, 91.3%, 95.6% and 56.8%, respectively.

Tables 3 and 4 show the patients' clinical characteristics and presenting symptoms by group. In these tables, 1 person was excluded from the FN group for developing bacterial pneumonia 72 hours after presenting with a high fever. No significant differences were found in age, sex, vaccination status and body temperature among the FN, TP and TN groups. The time from symptom onset to test was significantly earlier in the FN group compared to TP (11.4±10.9 vs. 22.0±17.3 hours, p=0.0010). However, no significant differences between these groups were found for the other factors and symptoms assessed. The FN and TN groups were compared in order to reveal diagnostic factors or symptoms. More patients presented with chills in the FN group (7/15 vs. 3/21, p=0.032). No other significant differences were found.

Combining the RIADT result and presence of chills increased the sensitivity and the NPV from 72.9% to 86.4% and 56.8% to 69.2%, respectively.

Table 1: Baseline patient characteristics

Patient characteristics		n (%)
Age (years)		
Median 30.5, Range 20-63		
	<30	37 (45.1)
	30 to 49	36 (43.9)
	≥50	9 (11.0)
Male gender (%)		35 (42.7)
Vaccination status (%)		41 (50.0)
Time to test from symptom onset (hours)		
Mean 18.9±17.3		
	<6	11 (13.4)
	6-12	14 (17.1)
	12-24	34 (41.5)
	24-48	13 (15.8)
	≥48	9 (11.0)
	Unknown	1 (1.2)

Table 2: RIADT (RapidTesta II) accuracy

		RIADT		Total
		A(+) and/or B(+)	(-)	
Verigene system	**Positive**	43	16	59
	Negative	2	21	23
Total		45	37	82

Prevalence: 72.0%
Sensitivity: 72.9%,
Specificity: 91.3%
Positive predictive value (PPV): 95.6%, Negative predictive value (NPV): 56.8

Table 3: Patient characteristics by group.

Patient characteristics		FN group (n=15*)	TP group (n=43)	TN group (n=21)	*p*-Value	
					FN vs. TP	FN vs. TN
Age in years	Median	31	30	29	0.642	0.753
	Range	20-57	21-62	23-63		
Age distribution (years)		**n (%)**	**n (%)**	**n (%)**		
	<30	6 (40.0)	19 (44.2)	11 (52.4)		
	30 to 49	6 (40.0)	20 (46.5)	8 (38.1)		
	≧50	3 (20.0)	4 (9.3)	2 (9.5)		
Male gender		7 (46.7)	17 (39.5)	9 (42.9)	0.629	0.821
Vaccination status		5 (33.3)	22 (51.2)	13 (61.9)	0.233	0.091
Time to test from symptom onset (hours)						
	Mean	11.4±10.9	22.0±17.3	15.7±16.8	0.0010 †	0.075
Time to test from symptom onset (hours)		**n (%)**	**n (%)**	**n (%)**		
	<6	3 (20.0)	4 (9.3)	2 (14.3)		
	6 to 12	6 (40.0)	6 (14.0)	2 (9.5)		
	12 to 24	5 (33.3)	16 (37.2)	11 (52.4)		
	24 to 48	0 (0.0)	11 (25.6)	2 (9.5)		
	≧48	1 (6.7)	6 (14.0)	2 (9.5)		
	unknown	0 (0.0)	0 (0.0)	1 (4.8)		

*Excluded one patient with bacterial pneumonia that developed after influenza viral infection.
† $p<0.05$
FN=False Negative, TP=True Positive, TN=True Negative.

Table 4: Symptoms and clinical characteristics at presentation.

Patient characteristics	FN group (n=15*)	TP group (n=43)	TN group (n=21)	*p*-Value	
				FN vs. TP	FN vs. TN
Body temperature (°C)					
Mean	38.2±0.8	38.0±0.7	37.6±0.8	0.785	0.994
Body temperature (°C)	**n (%)**	**n (%)**	**n (%)**		
T≦37.4°	2 (13.3)	8 (18.6)	9 (42.9)		
T=37.5 to 37.7	2 (13.3)	9 (20.9)	3 (14.3)		
T=37.8 to 38.0	3 (20.0)	6 (14.0)	4 (19.0)		
T≧38.1	8 (53.3)	20 (46.5)	5 (23.8)		
Symptoms					
Sore throat/pharyngeal erythema	11 (73.3)	32 (74.4)	14 (66.7)	0.934	0.669
Arthralgia/myalgia	6 (40.0)	22 (51.2)	10 (47.6)	0.456	0.650
Headache	7 (46.7)	13 (30.2)	6 (28.6)	0.249	0.265
Chills	7 (46.7)	16 (37.2)	3 (14.3)	0.519	0.032 †
Cough/septum	9 (60.0)	25 (58.1)	10 (47.6)	0.900	0.463
Nasal discharge	2 (13.3)	15 (34.9)	6 (28.6)	0.114	0.278

*Excluded one patient with bacterial pneumonia that developed after influenza viral infection.

† $p<0.05$

FN=False Negative, TP=True Positive, TN=True Negative.

DISCUSSION

RIADTs

Influenza virus infection is confirmed by virus isolation, viral nucleic acid detection (by RT-PCR, for example) or detecting a rising serum antibody titer in the acute and convalescent period.[19,21,23,24] But these tests take too much time and are too costly, and so are rarely used clinically in Japan. Before introduction of RIADTs to Japan in 1999, physicians diagnosed influenza by assessing clinical symptoms and epidemiological information.

RIADTs are widely used today, because they are simple, inexpensive, and require no special facilities, equipment or technology.[25] Use of RIADTs can decrease unnecessary blood tests, imaging studies or antibiotic use.

According to our RIADT correlative examination results for type A influenza, the sensitivity is 94.3% and specificity is 97.8%, and 87.0% and 100%, respectively, for type B influenza,. However, clinically, the sensitivity is relatively low, as was our result. Reported factors which lower the sensitivity are the following: type B influenza virus, pandemic 2009 influenza A (H1N1) virus, the timing of the test, use in adult patients, low fever, small amounts of sample, prior vaccination and poor sampling technique.[19,20]

Time from symptom onset to the test

In the current study, the mean time from symptom onset to test in the FN group was 11.4±10.9 hours, and 22.0±17.3 hours in the TP group. The FN group was tested significantly earlier than the TP group (p=0.001). This suggests that testing too early is a factor increasing false negative results, a finding which was consistent with a previous report.[19]

RIADTs are immunoassays using the antigen-antibody reaction, based on colloidal gold immunochromatography. The test results are checked visually. The RIADT used for this study, the RapidTesta FLU II, requires a 1×10^5 tissue-culture infective dose $(TCID)_{50}$ / mL for type A influenza, and 1.2×10^5 $(TCID)_{50}$/mL for type B influenza, to produce a positive result. The influenza virus proliferates in respiratory tract epithelial cells, and appears in respiratory secretions 24 hours before symptom onset. The peak of viral shedding is 24 hours after symptom onset, and then the virus load decreases rapidly.[26] In the current study, we assumed that the amount of virus in the FN group was less than that in the TP group.

Chills

A previously reported prospective cohort study revealed the relationship between chills and bacteremia: chills were divided into 3 categories of mild, moderate, and severe, and a greater degree of chills suggested a higher risk of bacteremia.[27] The report of a systematic review from 2004 regarding the clinical diagnosis of influenza states that the medical history and physical examination findings of rigor, fever and sweating are best for positive influenza diagnosis (likelihood ratios +7.2, + 4.0 and + 3.0, respectively).[28] In that review, chills were divided into rigor (shivering or severe chills) and chills (mild to moderate), and the likelihood ratio of chills was +1.1. In the current study, the FN group had more chills than did the TN group (p=0.0032). The presence of chills is supposed thought to be an indicator of influenza if the RIADT result is negative. We did not assess the degree of chills in the current study, so future research is required to determine which degree of chills indicates greater risk of influenza.

Limitations

The 82 subjects enrolled in the current study were relatively young and healthy. Elderly people tend to present with atypical symptoms and often have underlying primary illnesses. For this reason, a similar study that included elderly people would likely have different results.

The US Center for Disease Control and Prevention suggested that the pandemic 2009 influenza A (H1N1) virus would continue to spread for years to come, like a seasonal influenza virus.[29] The viruses detected in the current samples were almost all pandemic 2009 A (H1N1) or influenza B. The sensitivity of RIADTs for pandemic 2009 influenza A (H1N1) virus was previously reported to be a little lower than that for influenza A (H3N2) virus,[20] but in the current study, the sensitivity was equal for the two strains.

The reference or "gold" standard in this study was not viral culture or RT-PCR but analysis using the Verigene System RV+, which detects influenza viral nucleic acid. The sensitivity of this system for influenza A is 99.2% and the specificity is 90.1%. For influenza B, the sensitivity is 96.8% and the specificity is 98.5%.[21] A reference standard was applied.

CONCLUSIONS

Administration of an RIADT too early after symptom onset causes false negative results. Chills are thought to be an indicator of influenza virus infection even if the RIADT result is negative. Physicians should keep this information in mind when assessing possible influenza virus infections.

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Contributors

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CONFLICT OF INTEREST

None of the authors has conflict of interest with the submission.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	#1, #3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	#3-#4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	#6-#7
Objectives	3	State specific objectives, including any prespecified hypotheses	#7
Methods			
Study design	4	Present key elements of study design early in the paper	#7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	#7-#8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	#7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	#7-#8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	#7-#9
Bias	9	Describe any efforts to address potential sources of bias	#17
Study size	10	Explain how the study size was arrived at	#7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	#7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	#9
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	#9
		(b) Give reasons for non-participation at each stage	No
		(c) Consider use of a flow diagram	No
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	#9-#11
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	#10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	#15-#16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	#16-#17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	#17
Generalisability	21	Discuss the generalisability (external validity) of the study results	#16-#17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	#17-#18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT) : a cross-sectional study.

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Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT): a cross-sectional study.

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Key words: Influenza, Rapid influenza antigen detection test, Sensitivity and specificity, False negative, Symptoms influencing RIADT results, Chills, Cross-sectional study.

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ABSTRACT

[Objective] To evaluate the diagnostic accuracy of the rapid influenza antigen detection test (RIADT) and determine which symptoms are relevant to results.

[Design] Single-center, cross-sectional study.

[Setting] Primary care center, Tokyo, Japan.

[Participants] 82 consecutive outpatients presenting with upper respiratory symptoms and fever ≧37℃ at any time from symptom onset, between December 2010 and April 2011.

[Main Outcome Measures] Results of history and physical examination, including age, sex, temperature, time of test from symptom onset, vaccination record and current symptoms (sore throat, arthralgia and/or myalgia, headache, chills, cough and/or throat phlegm, nasal discharge) were recorded. The RIADT and a fully automated respiratory virus nucleic acid test (Verigene Respiratory Virus Plus; VRV), the latter being the gold standard, were performed. Patients were divided into 4 groups: False Negative (FN), RIADT- and VRV+; True Positive (TP), RIADT+ and VRV+; True Negative (TN), RIADT- and VRV-; and False Positive (FP), RIADT+ and VRV-. Groups were compared regarding age, sex, temperature, time of test from symptom onset, vaccination record, and symptoms.

[Key Results] RIADT sensitivity, specificity, positive predictive value and negative predictive value were 72.9% (95% CI, 61.5-84.2), 91.3% (79.7-102.8), 95.6% (89.5-101.6) and 56.8% (40.8-72.7), respectively. Time from symptom onset to test was shorter for the FN than the TP group (p=0.009). No significant differences were detected for the other factors assessed. Results revealed higher temperatures for FN than TN patients (p=0.043), and more FN than TN patients had chills (p=0.058).

[Conclusions] The RIADT sensitivity was low, due to early administration of the test. In the

epidemic season, the RIADT should not be used for suspected influenza until 12 hours after symptom onset. A positive RIADT firmly supports the influenza diagnosis; a negative result does not confirm its absence. High fever and chills might indicate influenza, but additional tests are sometimes necessary.

[298words]

ARTICLE SUMMARY

Strengths and limitations of this study

- Influenza is one of the most common diseases that general physicians see clinically. The RIADT is helpful for diagnosis, but its low sensitivity is sometimes misleading, resulting in under-diagnosis. We aimed to evaluate the diagnostic accuracy and characteristics of the RIADT and determine which symptoms are relevant to the results. Our findings may help general physicians when using the RIADT.
- Clinically, the sensitivity of RIADT is relatively low and the specificity is high; current results confirm this. Testing too early could be a factor increasing false negative results. For patients presenting with high fever and upper respiratory symptoms soon after onset, RIADT should not be used.
- The presence of high fever and chills may be helpful indicators of influenza, even if the RIADT result is negative. But additional examination is necessary for patients with symptoms inconsistent with influenza virus infection.
- The high specificity of the RIADT means that a positive result provides firm support for the diagnosis of influenza.
- The following limitations need to be acknowledged. First, the 82 subjects enrolled in the current study were relatively young and healthy. Second, the reference or "gold" standard in this study was not viral culture or reverse transcriptase-polymerase chain reaction (RT-PCR), but the Verigene System VRV, which detects influenza virus nucleic acid.

INTRODUCTION

Influenza is a rapid-onset systemic illness caused by the influenza virus; patients present with high fever, chills, cough, myalgia, sore throat and headache.[1, 2] Previously, the diagnosis was made from these symptoms.[3-6] However, since their introduction to Japan in 1999, rapid influenza antigen detection tests (RIADTs) using immunochromatography have dramatically changed the influenza diagnostic procedure.[7,8] Before the introduction of RIADTs and anti-influenza drugs, physicians told patients to stay home if they had no suspected complications. Now physicians use RIADTs to diagnose influenza and therefore can prescribe anti-viral drugs soon after symptom onset.[8-13] Making the distinction between the flu and other respiratory diseases serves to improve individual care management.[14-16] Detection of influenza virus can reduce inappropriate antibiotic use, guide anti-viral therapy, and decrease use of other laboratory studies and healthcare costs.[14] Currently, many RIADTs are available; their sensitivities and specificities have improved and their usefulness has been widely recognized.[17] RIADTs help physicians diagnose influenza during epidemics, but the RIADT results make it difficult to diagnose flu during periods of transition from epidemic to non-epidemic times, or when patients present with atypical symptoms.[17,18]

For example, when patients' flu-like symptoms are typical of influenza but the RIADT results are negative, whether or not the patient actually has influenza is unclear. Physicians may question whether samples were obtained correctly or whether the result is a false negative, and may hesitate to prescribe anti-influenza drugs.

According to a meta-analysis reported in 2012, the pooled RIADT sensitivity was 62.3% (95% CI, 57.9-66.6%), and specificity was 98.2% (95% CI, 97.5-98.7%).[19] Thus, the specificity is very high but the sensitivity is relatively low. Several factors affecting the results of

RIADTs have been reported, but some remain controversial.[19-20]

This study was designed to evaluate the diagnostic accuracy and characteristics of one RIADT, the RapidTesta FLUII (Sekisui Medical, Tokyo, Japan), and to determine which symptoms are associated with the results obtained. In addition, we sought to identify predictors of influenza for patients with false negative RIADT results.

METHODS

From December 2010 to April 2011, during the influenza epidemic season in Japan,[21] participants were enrolled in the Departments of General Medicine of Juntendo University Hospital and Juntendo University Nerima Hospital, both in Tokyo, Japan. Enrolled were consecutive cases who met the following inclusion criteria: adult patients presenting with any upper respiratory symptoms, and fever ≧37 °C at any time after symptom onset. All of the participants were physically examined and historical data, including age, sex, vaccination status, temperature and symptoms (sore throat, arthralgia and/or myalgia, headache, chills, cough and/or throat phlegm, and nasal discharge) were recorded, as was the time to test from symptom onset. Vaccination status indicated whether an influenza vaccine had been administered during that season before symptom onset. The temperature was taken on presentation by an outpatient physician. Symptoms recorded were those participants reported on presentation. Only a few patients had taken antipyretics and their temperature was around 36°C, but we could not analyze the data with regard to antipyretics use. All were tested by both the RIADT and a fully automated respiratory virus nucleic acid test, Verigene Respiratory Virus Plus (VRV) (NanoSphere, Chicago, IL, USA), the latter being the gold standard for this study. Based on the test results, patients were divided into 4 groups, as follows: False Negative (FN), RIADT- and

VRV+; True Positive (TP), RIADT+ and VRV+; True Negative (TN), RIADT- and VRV-; and False Positive (FP), RIADT+ and VRV-. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RIADT were calculated. In order to ascertain the cause of false-negative results and to find a predictor of influenza, comparisons were made between pairs of groups (FN vs. TP and then FN vs. TN) with respect to age, sex, vaccination status, time from symptom onset to test, temperature, and symptoms.

Laboratory Confirmation

Two nasopharyngeal specimens were collected from each patient by a physician, using sterile cotton swabs, following the procedure detailed in the RIADT manufacturer's package insert.

1. RIADT

RIADTs are immunoassays using the antigen-antibody reaction, based on colloidal gold immunochromatography. The test results are checked visually. The RIADT used for this study, the RapidTesta FLU II, requires a 1×10^5 tissue-culture infective dose $(TCID)_{50}$ per mL for type A influenza, and 1.2×10^5 $TCID_{50}$/mL for type B influenza, to produce a positive result.[22] RIADT was performed using one of the nasopharyngeal specimens, according to the manufacturer's instructions. In brief, samples were diluted in the medium immediately, and then dripped into the test device. Results were determined 15 minutes after the test start. When influenza A or B is present, an additional red line appears next to the control red line on the letter 'A' or 'B' indicated on the test device. The procedures were performed by outpatient physicians and residents who had been well-trained in the technique.

2. Fully automated respiratory virus nucleic acid test (VRV)

The second nasopharyngeal swab was immediately placed into Universal Viral Transport medium (UTM; Becton Dickinson, Tokyo, Japan) and tested by the Verigene System within 24 hours. The system uses a multiplexed microarray-based technology. A nucleic acid detection cartridge named Verigene Respiratory Virus Plus Nucleic Acid Test (VRV) was selected, which could detect the following influenza viruses: A (H1N1), A (H3N2), pandemic 2009 influenza A (H1N1), influenza B virus, and respiratory syncytial virus (A, B). This system was approved by the American Food and Drug Administration (FDA) in May 2009.[23]

Specimens were tested by Verigene test according to the manufacturer's instructions by the physician attending the study patients. Briefly, the test cartridge was pre-loaded with wash solutions, oligonucleotide probe solution and signal amplification solution. The extraction tray, amplification tray and test cartridge were then loaded onto the Verigene System. Following the addition of 200 microliters of UTM containing material expressed from the nasopharyngeal swabs, the analysis began; this consisted of a programmed, totally automated extraction, reverse transcriptase-polymerase chain reaction (RT-PCR), and hybridization sequence. The final readout of the microarray was made by the insertion of the slide array into a reader.[24] The result for each virus type, 'Detected' or 'Not detected,' was displayed on a monitor. Approximately 2.5 hours was required from sample procurement to final readout.

Specimen collection was performed by one of 5 physicians, who also read the results. These physicians were trained by an instructor from the manufacturer before the study commenced.

Statistical Analysis

The sensitivity, specificity, PPV and NPV of the RIADT used were determined using standard methods. Statistical analyses were performed using statistical software package, STATA SE 12 (StataCorp LP, College Station, TX, USA). Continuous variables (age, the time from symptom onset, and temperature) were analyzed by the Wilcoxon rank sum test, and the Fisher's exact test was used for comparing patient sex, vaccination status and symptoms. Significance was assigned to results having P values <0.05, and borderline significance was assigned to P values >0.05 and <0.10.

Ethical Statement

This study was reviewed and approved by the internal review boards of Juntendo University Hospital (approval number 22-290) and Juntendo Nerima Hospital (approval number 10-26).

RESULTS

A total of 82 consecutive patients meeting eligibility criteria were enrolled from December 2010 to April 2011 (Juntendo University Hospital: 37 patients; Juntendo University Nerima Hospital: 45 patients). There was no selection discretion on the part of the attending physicians. Table 1 shows characteristics for all patients. The median age was 30.5 (range 20-63) years, and 42.7% (35/82) were men. During the 2010/2011 flu season, 48.8% (40/82) were vaccinated for influenza. The average time from symptom onset to diagnostic test was 18.9±17.2 hours; 13.4% (11/82) came to the hospital within 6 hours from symptom onset, and 72.0% (59/82) within 24 hours.

Table 1: Baseline patient characteristics

Patient characteristics		n (%)
Age (years)		
Median 30.5, Range 20-63		
	<30	37 (45.1)
	30 to 49	36 (43.9)
	≥50	9 (11.0)
Male sex (%)		35 (42.7)
Vaccination status (%)		41 (50.0)
Time to test from symptom onset (hours)		
Mean 18.9±17.3		
	<6	11 (13.4)
	6-12	14 (17.1)
	12-24	34 (41.5)
	24-48	13 (15.8)
	≥48	9 (11.0)
	Unknown	1 (1.2)

Table 2 shows the RIADT and VRV results as well as the RIADT accuracy. By RIADT results, 54.9% (45/82) of patients were positive and the other 45.1% (37/82) were negative. By VRV results, 72.0% (59/82) were positive and the other 28.0% (23/82) were negative. Dividing them into 4 groups, the numbers in each were: FN, 16; TP, 43; TN, 21; and FP, 2. The prevalence of influenza A or B virus infection was 72.0%. When the Verigene VRV test was used as the gold standard, the RIADT sensitivity, specificity, PPV and NPV were 72.9% (95% CI, 61.5-84.2), 91.3% (79.7-102.8), 95.6% (89.5-101.6) and 56.8% (40.8-72.7), respectively.

Table 2: RIADT (RapidTesta II) accuracy

		RIADT		Total
		A(+) and/or B(+)	(-)	
Verigene test (VRV)	**Positive**	43	16	59
	Negative	2	21	23
Total		45	37	82

Prevalence: 72.0%
Sensitivity: 72.9% (95% CI, 61.5-84.2)
Specificity: 91.3% (95% CI, 79.7-102.8)
Positive predictive value (PPV): 95.6% (95% CI, 89.5-101.6)
Negative predictive value (NPV): 56.8% (95% CI, 40.8-72.7)

Tables 3 and 4 show the patients' clinical characteristics and presenting symptoms by group. One patient was excluded from the FN group due to development of bacterial pneumonia 72 hours after presenting with a high fever. No significant differences were found in age, sex and vaccination status among the FN, TP and TN groups. The time from symptom onset to test was

significantly earlier in the FN compared to the TP group (11.4±10.9 vs. 22.0±17.3 hours, p=0.009). However, no significant differences between these groups were found for the other factors and symptoms assessed. The FN and TN groups were compared in order to reveal any differences in diagnostic factors or symptoms. The temperature of the FN negative group was higher than the TN group (38.2±0.8 vs. 37.6±0.8, p=0.043). More patients presented with chills in the FN group (7/15 vs. 3/21, p=0.058: borderline significance). No other significant differences were found in symptoms.

Combining the RIADT result and presence of temperature ≧37.8 °C or chills increased the sensitivity and the NPV from 72.9% to 96.6% (95% CI, 92.0-101.2%) and from 56.8% to 90.5% (77.9-103.0%), respectively. The specificity and the PPV were 82.6% (67.1-98.1%) and 93.4% (87.2-99.7%), respectively.

Table 3: Patient characteristics by group.

Patient characteristics		FN group (n=15*)	TP group (n=43)	TN group (n=21)	*p*-Value	
					FN vs. TP	FN vs. TN
Age in years	Median	31	30	29	0.880	0.653
	Range	20-57	21-62	23-63		
Age distribution (years)		**n (%)**	**n (%)**	**n (%)**		
	<30	6 (40.0)	19 (44.2)	11 (52.4)		
	30 to 49	6 (40.0)	20 (46.5)	8 (38.1)		
	≧50	3 (20.0)	4 (9.3)	2 (9.5)		
Male sex		7 (46.7)	17 (39.5)	9 (42.9)	0.763	1.000
Vaccination status		5 (33.3)	22 (51.2)	13 (61.9)	0.368	0.176
Time to test from symptom onset (hours)						
	Mean	11.4±10.9	22.0±17.3	15.7±16.8	0.009 †	0.449
Time to test from symptom onset (hours)		**n (%)**	**n (%)**	**n (%)**		
	<6	3 (20.0)	4 (9.3)	2 (14.3)		
	6 to 12	6 (40.0)	6 (14.0)	2 (9.5)		
	12 to 24	5 (33.3)	16 (37.2)	11 (52.4)		
	24 to 48	0 (0.0)	11 (25.6)	2 (9.5)		
	≧48	1 (6.7)	6 (14.0)	2 (9.5)		
	unknown	0 (0.0)	0 (0.0)	1 (4.8)		

*Excluded one patient with bacterial pneumonia that developed after influenza virus infection.
†p<0.05
FN=False Negative, TP=True Positive, TN=True Negative.

Table 4: Symptoms and clinical characteristics at presentation.

Patient characteristics	FN group (n=15*)	TP group (n=43)	TN group (n=21)	*p*-Value	
				FN vs. TP	FN vs. TN
Temperature (°C)					
Mean	38.2±0.8	38.0±0.7	37.6±0.8	0.593	0.043 †
Temperature (°C)	**n (%)**	**n (%)**	**n (%)**		
T≦37.4°	2 (13.3)	8 (18.6)	9 (42.9)		
T=37.5 to 38.0	5 (33.3)	15 (54.9)	7 (33.3)		
T≧38.1	8 (53.3)	20 (46.5)	5 (23.8)		
Symptoms					
Sore throat	11 (73.3)	32 (74.4)	14 (66.7)	1.000	0.729
Arthralgia/myalgia	6 (40.0)	22 (51.2)	10 (47.6)	0.554	0.741
Headache	7 (46.7)	13 (30.2)	6 (28.6)	0.345	0.310
Chills	7 (46.7)	16 (37.2)	3 (14.3)	0.553	0.058 ‡
Cough/throat phlegm	9 (60.0)	25 (58.1)	10 (47.6)	1.000	0.516
Nasal discharge	2 (13.3)	15 (34.9)	6 (28.6)	0.118	0.424

*Excluded one patient with bacterial pneumonia that developed after influenza viral infection.

†$p<0.05$

‡$0.05<p<0.1$

FN=False Negative, TP=True Positive, TN=True Negative.

DISCUSSION

RIADTs

Influenza virus infection is confirmed by virus isolation, viral nucleic acid detection (by RT-PCR, for example) or detecting a rising serum antibody titer in the acute and convalescent period.[19,23,25,26] However, these tests take much time and are costly, and so they are rarely used clinically in Japan.[24] Before introduction of RIADTs to Japan in 1999, physicians diagnosed influenza by assessing clinical symptoms and epidemiological information.

RIADTs are widely used today, because they are simple, inexpensive, and require no special facilities, equipment or technology.[27] Use of RIADTs can decrease unnecessary blood tests, imaging studies and antibiotic use.

According to our RIADT correlative examination results for type A influenza, the sensitivity is 94.3% and specificity is 97.8%, and the values for type B influenza are 87.0% and 100%, respectively, referencing results of virus culture and PCR.[22] Clinically, the specificity is high but the sensitivity is low. Reported factors which lower the sensitivity are the following: type B influenza virus, pandemic 2009 influenza A (H1N1) virus, the timing of the test, use in adult patients, low fever, small amounts of sample, prior vaccination and poor sampling technique.[19,20]

Time from symptom onset to the test

In the current study, the mean time from symptom onset to test in the FN group was 11.4±10.9 hours, and 22.0±17.3 hours in the TP group. The FN group was tested significantly earlier than the TP group (p=0.009). This suggests that testing too early is a factor increasing

false negative results, a finding that is consistent with a previous report.[19]

The RIADT used for this study, the RapidTesta FLU II, requires a 1×10^5 tissue-culture infective dose $(TCID)_{50}$/mL for type A influenza, and 1.2×10^5 $TCID_{50}$/mL for type B influenza, to produce a positive result. The influenza virus proliferates in respiratory tract epithelial cells, and appears in respiratory secretions 24 hours before symptom onset. The peak of viral shedding is 24 hours after symptom onset, and then the virus load decreases rapidly.[28] In the current study, we assumed that the amount of virus in the FN group was less than that in the TP group.

Symptoms

The report of a systematic review from 2004 regarding the clinical diagnosis of influenza states that the medical history and physical examination findings of rigor, fever and sweating are best for positive influenza diagnosis (likelihood ratios +7.2, + 4.0 and + 3.0, respectively).[28] Fever was defined as a temperature ≧37.8 °C or higher. [2] In the current study, the temperature of the FN group was significantly higher than that of the TN group (38.2±0.8 vs. 37.6±0.8, p=0.043), but no significant difference of temperature was detected between the FN and TP groups (38.2±0.8 vs. 38.0±0.7, p=0.593). This indicates that the temperature of influenza patients is relatively high. It should be noted that, even if RIADT is negative, it is possible that patients presenting with high fever have influenza.

A previously reported prospective cohort study revealed the relationship between chills and bacteremia: chills were divided into 4 categories of none, mild, moderate, and severe, and a greater degree of chills suggested a higher risk of bacteremia.[29] We did not categorize chills, but found that the FN group had more chills than did the TN group. The p value of 0.058, as 'borderline significant', was caused by having an insufficient number of participants. The

presence of chills is also thought to be an indicator of influenza if the RIADT result is negative.

The positive RIADT result gives physicians firm support for the diagnosis of influenza because of the high specificity of this test, but a negative result does not confirm its absence. Presence of high fever and chills are indicative of influenza, but if patients' presenting symptoms are inconsistent with influenza or acute upper respiratory infection, additional studies are necessary to make an accurate diagnosis. High fever with moderate to severe chills sometimes indicates bacteremia or other bacterial infection.[29]

Limitations

The 82 subjects enrolled in the current study were relatively young and healthy. This is because our hospitals are located in central Tokyo and most patients are young or middle-aged healthy workers. We did not exclude elderly patients or patients with co-morbid diseases. Elderly people tend to present with atypical symptoms and often have underlying primary illnesses. Also, because almost all of our patients are adults, pediatric patients were not included in this study. This group has distinctive symptoms, and the RIADT has the highest sensitivity in these patients. For this reason, applying similar research methods to different age groups may produce different results.

The US Center for Disease Control and Prevention suggested that the 2009 pandemic influenza A (H1N1) virus would continue to spread for years to come as a seasonal influenza virus.[31] The viruses detected in the current samples were almost all 2009 pandemic influenza A (H1N1) or B. The sensitivity of RIADTs for 2009 pandemic influenza A (H1N1) virus was previously reported to be a little lower than that for influenza A (H3N2) virus,[20] but in the current study, the sensitivity was equal for the two strains.

The reference, or "gold," standard in this study was not viral culture or RT-PCR but analysis using the Verigene test VRV, which detects influenza viral nucleic acid. When direct fluorescent antibody identification and viral culture were used as the gold standards, the sensitivity of the VRV system used in this study for influenza A was 98.7% (95% CI, 96.8-99.5%) and the specificity was 93.2% (95% CI, 91.1-99.9%). For influenza B, the sensitivity was 100% (95% CI, 91.8-100%) and the specificity was 99.7% (95% CI, 99.1-99.9%).[32] A reference standard was applied.

CONCLUSIONS

Consistent with previous reports, the sensitivity of the RIADT used in this study was low, due to early administration of the test. Administration of an RIADT too early after symptom onset causes false negative results. In the influenza epidemic season, practitioners should not use RIADT for patients with upper respiratory symptoms and high fever for at least 12 hours after onset. A positive RIADT result after this gives the physician firm support for a diagnosis of influenza. A negative RIADT result does not mean 'no influenza'. Presence of high fever and chills might predict influenza, but additional tests are necessary for patients with specific symptoms inconsistent with a diagnosis of influenza virus infection.

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Contributors

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CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest regarding the publication of this research.

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Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT): a cross-sectional study.

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ABSTRACT

[Objective] To evaluate the diagnostic accuracy of the rapid influenza antigen detection test (RIADT) and determine which symptoms are relevant to results.

[Design] Single-center, cross-sectional study.

[Setting] Primary care center, Tokyo, Japan.

[Participants] 82 consecutive outpatients presenting with upper respiratory symptoms and fever ≧37℃ at any time from symptom onset, between December 2010 and April 2011.

[Main Outcome Measures] Results of history and physical examination, including age, sex, temperature, time of test from symptom onset, vaccination record and current symptoms (sore throat, arthralgia and/or myalgia, headache, chills, cough and/or throat phlegm, nasal discharge) were recorded. The RIADT and a fully automated respiratory virus nucleic acid test (Verigene Respiratory Virus Plus; VRV), the latter being the gold standard, were performed. Patients were divided into 4 groups: False Negative (FN), RIADT- and VRV+; True Positive (TP), RIADT+ and VRV+; True Negative (TN), RIADT- and VRV-; and False Positive (FP), RIADT+ and VRV-. Groups were compared regarding age, sex, temperature, time of test from symptom onset, vaccination record, and symptoms.

[Key Results] RIADT sensitivity, specificity, positive predictive value and negative predictive value were 72.9% (95% CI, 61.5-84.2), 91.3% (79.7-102.8), 95.6% (89.5-101.6) and 56.8% (40.8-72.7), respectively. Time from symptom onset to test was shorter for the FN than the TP group (p=0.009). No significant differences were detected for the other factors assessed. Results revealed higher temperatures for FN than TN patients (p=0.043), and more FN than TN patients had chills (p=0.058).

[Conclusions] The RIADT sensitivity was low, due to early administration of the test. In the

epidemic season, the RIADT should not be used for suspected influenza until 12 hours after symptom onset. A positive RIADT firmly supports the influenza diagnosis; a negative result does not confirm its absence. High fever and chills might indicate influenza, but additional tests are sometimes necessary.

[298words]

ARTICLE SUMMARY

Strengths and limitations of this study

- Influenza is one of the most common diseases that general physicians see clinically. The RIADT is helpful for diagnosis, but its low sensitivity is sometimes misleading, resulting in under-diagnosis. We aimed to evaluate the diagnostic accuracy and characteristics of the RIADT and determine which symptoms are relevant to the results. Our findings may help general physicians when using the RIADT.
- Clinically, the sensitivity of RIADT is relatively low and the specificity is high; current results confirm this. Testing too early could be a factor increasing false negative results. For patients presenting with high fever and upper respiratory symptoms soon after onset, RIADT should not be used.
- The presence of high fever and chills may be helpful indicators of influenza, even if the RIADT result is negative. But additional examination is necessary for patients with symptoms inconsistent with influenza virus infection.
- The high specificity of the RIADT means that a positive result provides firm support for the diagnosis of influenza.
- The following limitations need to be acknowledged. First, the 82 subjects enrolled in the current study were relatively young and healthy. Second, the reference or "gold" standard in this study was not viral culture or reverse transcriptase-polymerase chain reaction (RT-PCR), but the Verigene System VRV, which detects influenza virus nucleic acid.

INTRODUCTION

Influenza is a rapid-onset systemic illness caused by the influenza virus; patients present with high fever, chills, cough, myalgia, sore throat and headache.[1, 2] Previously, the diagnosis was made from these symptoms.[3-6] However, since their introduction to Japan in 1999, rapid influenza antigen detection tests (RIADTs) using immunochromatography have dramatically changed the influenza diagnostic procedure.[7,8] Before the introduction of RIADTs and anti-influenza drugs, physicians told patients to stay home if they had no suspected complications. Now physicians use RIADTs to diagnose influenza and therefore can prescribe anti-viral drugs soon after symptom onset.[8-13] Making the distinction between the flu and other respiratory diseases serves to improve individual care management.[14-16] Detection of influenza virus can reduce inappropriate antibiotic use, guide anti-viral therapy, and decrease use of other laboratory studies and healthcare costs.[14] Currently, many RIADTs are available; their sensitivities and specificities have improved and their usefulness has been widely recognized.[17] RIADTs help physicians diagnose influenza during epidemics, but the RIADT results make it difficult to diagnose flu during periods of transition from epidemic to non-epidemic times, or when patients present with atypical symptoms.[17,18]

For example, when patients' flu-like symptoms are typical of influenza but the RIADT results are negative, whether or not the patient actually has influenza is unclear. Physicians may question whether samples were obtained correctly or whether the result is a false negative, and may hesitate to prescribe anti-influenza drugs.

According to a meta-analysis reported in 2012, the pooled RIADT sensitivity was 62.3% (95% CI, 57.9-66.6%), and specificity was 98.2% (95% CI, 97.5-98.7%).[19] Thus, the specificity is very high but the sensitivity is relatively low. Several factors affecting the results of

RIADTs have been reported, but some remain controversial.[19-20]

This study was designed to evaluate the diagnostic accuracy and characteristics of one RIADT, the RapidTesta FLUII (Sekisui Medical, Tokyo, Japan), and to determine which symptoms are associated with the results obtained. In addition, we sought to identify predictors of influenza for patients with false negative RIADT results.

METHODS

From December 2010 to April 2011, during the influenza epidemic season in Japan,[21] participants were enrolled in the Departments of General Medicine of Juntendo University Hospital and Juntendo University Nerima Hospital, both in Tokyo, Japan. Enrolled were consecutive cases who met the following inclusion criteria: adult patients presenting with any upper respiratory symptoms, and fever ≧37 °C at any time after symptom onset. All of the participants were physically examined and historical data, including age, sex, vaccination status, temperature and symptoms (sore throat, arthralgia and/or myalgia, headache, chills, cough and/or throat phlegm, and nasal discharge) were recorded, as was the time to test from symptom onset. Vaccination status indicated whether an influenza vaccine had been administered during that season before symptom onset. The temperature was taken on presentation by an outpatient physician. Symptoms recorded were those participants reported on presentation. Only a few patients had taken antipyretics and their temperature was around 36°C, but we could not analyze the data with regard to antipyretics use. All were tested by both the RIADT and a fully automated respiratory virus nucleic acid test, Verigene Respiratory Virus Plus (VRV) (NanoSphere, Chicago, IL, USA), the latter being the gold standard for this study. Based on the test results, patients were divided into 4 groups, as follows: False Negative (FN), RIADT- and

VRV+; True Positive (TP), RIADT+ and VRV+; True Negative (TN), RIADT- and VRV-; and False Positive (FP), RIADT+ and VRV-. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RIADT were calculated. In order to ascertain the cause of false-negative results and to find a predictor of influenza, comparisons were made between pairs of groups (FN vs. TP and then FN vs. TN) with respect to age, sex, vaccination status, time from symptom onset to test, temperature, and symptoms.

Laboratory Confirmation

Two nasopharyngeal specimens were collected from each patient by a physician, using sterile cotton swabs, following the procedure detailed in the RIADT manufacturer's package insert.

1. RIADT

RIADTs are immunoassays using the antigen-antibody reaction, based on colloidal gold immunochromatography. The test results are checked visually. The RIADT used for this study, the RapidTesta FLU II, requires a 1×10^5 tissue-culture infective dose $(TCID)_{50}$ per mL for type A influenza, and 1.2×10^5 $TCID_{50}$/mL for type B influenza, to produce a positive result.[22] RIADT was performed using one of the nasopharyngeal specimens, according to the manufacturer's instructions. In brief, samples were diluted in the medium immediately, and then dripped into the test device. Results were determined 15 minutes after the test start. When influenza A or B is present, an additional red line appears next to the control red line on the letter 'A' or 'B' indicated on the test device. The procedures were performed by outpatient physicians and residents who had been well-trained in the technique.

2. Fully automated respiratory virus nucleic acid test (VRV)

The second nasopharyngeal swab was immediately placed into Universal Viral Transport medium (UTM; Becton Dickinson, Tokyo, Japan) and tested by the Verigene System within 24 hours. The system uses a multiplexed microarray-based technology. A nucleic acid detection cartridge named Verigene Respiratory Virus Plus Nucleic Acid Test (VRV) was selected, which could detect the following influenza viruses: A (H1N1), A (H3N2), pandemic 2009 influenza A (H1N1), influenza B virus, and respiratory syncytial virus (A, B). This system was approved by the American Food and Drug Administration (FDA) in May 2009.[23]

Specimens were tested by Verigene test according to the manufacturer's instructions by the physician attending the study patients. Briefly, the test cartridge was pre-loaded with wash solutions, oligonucleotide probe solution and signal amplification solution. The extraction tray, amplification tray and test cartridge were then loaded onto the Verigene System. Following the addition of 200 microliters of UTM containing material expressed from the nasopharyngeal swabs, the analysis began; this consisted of a programmed, totally automated extraction, reverse transcriptase-polymerase chain reaction (RT-PCR), and hybridization sequence. The final readout of the microarray was made by the insertion of the slide array into a reader.[24] The result for each virus type, 'Detected' or 'Not detected,' was displayed on a monitor. Approximately 2.5 hours was required from sample procurement to final readout.

Specimen collection was performed by one of 5 physicians, who also read the results. These physicians were trained by an instructor from the manufacturer before the study commenced.

Statistical Analysis

The sensitivity, specificity, PPV and NPV of the RIADT used were determined using standard methods. Statistical analyses were performed using statistical software package, STATA SE 12 (StataCorp LP, College Station, TX, USA). Continuous variables (age, the time from symptom onset, and temperature) were analyzed by the Wilcoxon rank sum test, and the Fisher's exact test was used for comparing patient sex, vaccination status and symptoms. Significance was assigned to results having P values <0.05, and borderline significance was assigned to P values >0.05 and <0.10.

Ethical Statement

This study was reviewed and approved by the internal review boards of Juntendo University Hospital (approval number 22-290) and Juntendo Nerima Hospital (approval number 10-26).

RESULTS

A total of 82 consecutive patients meeting eligibility criteria were enrolled from December 2010 to April 2011 (Juntendo University Hospital: 37 patients; Juntendo University Nerima Hospital: 45 patients). There was no selection discretion on the part of the attending physicians. Table 1 shows characteristics for all patients. The median age was 30.5 (range 20-63) years, and 42.7% (35/82) were men. During the 2010/2011 flu season, 48.8% (40/82) were vaccinated for influenza. The average time from symptom onset to diagnostic test was 18.9±17.2 hours; 13.4% (11/82) came to the hospital within 6 hours from symptom onset, and 72.0% (59/82) within 24 hours.

Table 1: Baseline patient characteristics

Patient characteristics		n (%)
Age (years)		
Median 30.5, Range 20-63		
	<30	37 (45.1)
	30 to 49	36 (43.9)
	≥50	9 (11.0)
Male sex (%)		35 (42.7)
Vaccination status (%)		41 (50.0)
Time to test from symptom onset (hours)		
Mean 18.9±17.3		
	<6	11 (13.4)
	6-12	14 (17.1)
	12-24	34 (41.5)
	24-48	13 (15.8)
	≥48	9 (11.0)
	Unknown	1 (1.2)

Table 2 shows the RIADT and VRV results as well as the RIADT accuracy. By RIADT results, 54.9% (45/82) of patients were positive and the other 45.1% (37/82) were negative. By VRV results, 72.0% (59/82) were positive and the other 28.0% (23/82) were negative. Dividing them into 4 groups, the numbers in each were: FN, 16; TP, 43; TN, 21; and FP, 2. The prevalence of influenza A or B virus infection was 72.0%. When the Verigene VRV test was used as the gold standard, the RIADT sensitivity, specificity, PPV and NPV were 72.9% (95% CI, 61.5-84.2), 91.3% (79.7-102.8), 95.6% (89.5-101.6) and 56.8% (40.8-72.7), respectively.

Table 2: RIADT (RapidTesta II) accuracy

		RIADT		Total
		A(+) and/or B(+)	(-)	
Verigene test (VRV)	**Positive**	43	16	59
	Negative	2	21	23
Total		45	37	82

Prevalence: 72.0%
Sensitivity: 72.9% (95% CI, 61.5-84.2)
Specificity: 91.3% (95% CI, 79.7-102.8)
Positive predictive value (PPV): 95.6% (95% CI, 89.5-101.6)
Negative predictive value (NPV): 56.8% (95% CI, 40.8-72.7)

Tables 3 and 4 show the patients' clinical characteristics and presenting symptoms by group. One patient was excluded from the FN group due to development of bacterial pneumonia 72 hours after presenting with a high fever. No significant differences were found in age, sex and vaccination status among the FN, TP and TN groups. The time from symptom onset to test was

significantly earlier in the FN compared to the TP group (11.4±10.9 vs. 22.0±17.3 hours, p=0.009). However, no significant differences between these groups were found for the other factors and symptoms assessed. The FN and TN groups were compared in order to reveal any differences in diagnostic factors or symptoms. The temperature of the FN negative group was higher than the TN group (38.2±0.8 vs. 37.6±0.8, p=0.043). More patients presented with chills in the FN group (7/15 vs. 3/21, p=0.058: borderline significance). No other significant differences were found in symptoms.

Combining the RIADT result and presence of temperature ≧37.8 °C or chills increased the sensitivity and the NPV from 72.9% to 96.6% (95% CI, 92.0-101.2%) and from 56.8% to 90.5% (77.9-103.0%), respectively. The specificity and the PPV were 82.6% (67.1-98.1%) and 93.4% (87.2-99.7%), respectively.

Table 3: Patient characteristics by group.

Patient characteristics		FN group (n=15*)	TP group (n=43)	TN group (n=21)	*p*-Value	
					FN vs. TP	FN vs. TN
Age in years	Median	31	30	29	0.880	0.653
	Range	20-57	21-62	23-63		
Age distribution (years)		**n (%)**	**n (%)**	**n (%)**		
	<30	6 (40.0)	19 (44.2)	11 (52.4)		
	30 to 49	6 (40.0)	20 (46.5)	8 (38.1)		
	≧50	3 (20.0)	4 (9.3)	2 (9.5)		
Male sex		7 (46.7)	17 (39.5)	9 (42.9)	0.763	1.000
Vaccination status		5 (33.3)	22 (51.2)	13 (61.9)	0.368	0.176
Time to test from symptom onset (hours)						
	Mean	11.4±10.9	22.0±17.3	15.7±16.8	0.009 †	0.449
Time to test from symptom onset (hours)		**n (%)**	**n (%)**	**n (%)**		
	<6	3 (20.0)	4 (9.3)	2 (14.3)		
	6 to 12	6 (40.0)	6 (14.0)	2 (9.5)		
	12 to 24	5 (33.3)	16 (37.2)	11 (52.4)		
	24 to 48	0 (0.0)	11 (25.6)	2 (9.5)		
	≧48	1 (6.7)	6 (14.0)	2 (9.5)		
	unknown	0 (0.0)	0 (0.0)	1 (4.8)		

*Excluded one patient with bacterial pneumonia that developed after influenza virus infection.
†p<0.05
FN=False Negative, TP=True Positive, TN=True Negative.

Table 4: Symptoms and clinical characteristics at presentation.

Patient characteristics	FN group (n=15*)	TP group (n=43)	TN group (n=21)	*p*-Value FN vs. TP	*p*-Value FN vs. TN
Temperature (°C)					
Mean	38.2±0.8	38.0±0.7	37.6±0.8	0.593	0.043 †
Temperature (°C)	**n (%)**	**n (%)**	**n (%)**		
T≦37.4°	2 (13.3)	8 (18.6)	9 (42.9)		
T=37.5 to 38.0	5 (33.3)	15 (54.9)	7 (33.3)		
T≧38.1	8 (53.3)	20 (46.5)	5 (23.8)		
Symptoms					
Sore throat	11 (73.3)	32 (74.4)	14 (66.7)	1.000	0.729
Arthralgia/myalgia	6 (40.0)	22 (51.2)	10 (47.6)	0.554	0.741
Headache	7 (46.7)	13 (30.2)	6 (28.6)	0.345	0.310
Chills	7 (46.7)	16 (37.2)	3 (14.3)	0.553	0.058 ‡
Cough/throat phlegm	9 (60.0)	25 (58.1)	10 (47.6)	1.000	0.516
Nasal discharge	2 (13.3)	15 (34.9)	6 (28.6)	0.118	0.424

*Excluded one patient with bacterial pneumonia that developed after influenza viral infection.

†$p<0.05$

‡$0.05<p<0.1$

FN=False Negative, TP=True Positive, TN=True Negative.

DISCUSSION

RIADTs

Influenza virus infection is confirmed by virus isolation, viral nucleic acid detection (by RT-PCR, for example) or detecting a rising serum antibody titer in the acute and convalescent period.[19,23,25,26] However, these tests take much time and are costly, and so they are rarely used clinically in Japan.[24] Before introduction of RIADTs to Japan in 1999, physicians diagnosed influenza by assessing clinical symptoms and epidemiological information.

RIADTs are widely used today, because they are simple, inexpensive, and require no special facilities, equipment or technology.[27] Use of RIADTs can decrease unnecessary blood tests, imaging studies and antibiotic use.

According to our RIADT correlative examination results for type A influenza, the sensitivity is 94.3% and specificity is 97.8%, and the values for type B influenza are 87.0% and 100%, respectively, referencing results of virus culture and PCR.[22] Clinically, the specificity is high but the sensitivity is low. Reported factors which lower the sensitivity are the following: type B influenza virus, pandemic 2009 influenza A (H1N1) virus, the timing of the test, use in adult patients, low fever, small amounts of sample, prior vaccination and poor sampling technique.[19,20]

Time from symptom onset to the test

In the current study, the mean time from symptom onset to test in the FN group was 11.4±10.9 hours, and 22.0±17.3 hours in the TP group. The FN group was tested significantly earlier than the TP group ($p=0.009$). This suggests that testing too early is a factor increasing

false negative results, a finding that is consistent with a previous report.[19]

The RIADT used for this study, the RapidTesta FLU II, requires a 1×10^5 tissue-culture infective dose $(TCID)_{50}$/mL for type A influenza, and 1.2×10^5 $TCID_{50}$/mL for type B influenza, to produce a positive result. The influenza virus proliferates in respiratory tract epithelial cells, and appears in respiratory secretions 24 hours before symptom onset. The peak of viral shedding is 24 hours after symptom onset, and then the virus load decreases rapidly.[28] In the current study, we assumed that the amount of virus in the FN group was less than that in the TP group.

Symptoms

The report of a systematic review from 2004 regarding the clinical diagnosis of influenza states that the medical history and physical examination findings of rigor, fever and sweating are best for positive influenza diagnosis (likelihood ratios +7.2, + 4.0 and + 3.0, respectively).[28] Fever was defined as a temperature ≧37.8 °C or higher. [2] In the current study, the temperature of the FN group was significantly higher than that of the TN group (38.2±0.8 vs. 37.6±0.8, p=0.043), but no significant difference of temperature was detected between the FN and TP groups (38.2±0.8 vs. 38.0±0.7, p=0.593). This indicates that the temperature of influenza patients is relatively high. It should be noted that, even if RIADT is negative, it is possible that patients presenting with high fever have influenza.

A previously reported prospective cohort study revealed the relationship between chills and bacteremia: chills were divided into 4 categories of none, mild, moderate, and severe, and a greater degree of chills suggested a higher risk of bacteremia.[29] We did not categorize chills, but found that the FN group had more chills than did the TN group. The p value of 0.058, as 'borderline significant', was caused by having an insufficient number of participants. The

presence of chills is also thought to be an indicator of influenza if the RIADT result is negative.

The positive RIADT result gives physicians firm support for the diagnosis of influenza because of the high specificity of this test, but a negative result does not confirm its absence. Presence of high fever and chills are indicative of influenza, but if patients' presenting symptoms are inconsistent with influenza or acute upper respiratory infection, additional studies are necessary to make an accurate diagnosis. High fever with moderate to severe chills sometimes indicates bacteremia or other bacterial infection.[29]

Limitations

The 82 subjects enrolled in the current study were relatively young and healthy. This is because our hospitals are located in central Tokyo and most patients are young or middle-aged healthy workers. We did not exclude elderly patients or patients with co-morbid diseases. Elderly people tend to present with atypical symptoms and often have underlying primary illnesses. Also, because almost all of our patients are adults, pediatric patients were not included in this study. This group has distinctive symptoms, and the RIADT has the highest sensitivity in these patients. For this reason, applying similar research methods to different age groups may produce different results.

The US Center for Disease Control and Prevention suggested that the 2009 pandemic influenza A (H1N1) virus would continue to spread for years to come as a seasonal influenza virus.[31] The viruses detected in the current samples were almost all 2009 pandemic influenza A (H1N1) or B. The sensitivity of RIADTs for 2009 pandemic influenza A (H1N1) virus was previously reported to be a little lower than that for influenza A (H3N2) virus,[20] but in the current study, the sensitivity was equal for the two strains.

The reference, or "gold," standard in this study was not viral culture or RT-PCR but analysis using the Verigene test VRV, which detects influenza viral nucleic acid. When direct fluorescent antibody identification and viral culture were used as the gold standards, the sensitivity of the VRV system used in this study for influenza A was 98.7% (95% CI, 96.8-99.5%) and the specificity was 93.2% (95% CI, 91.1-99.9%). For influenza B, the sensitivity was 100% (95% CI, 91.8-100%) and the specificity was 99.7% (95% CI, 99.1-99.9%).[32] A reference standard was applied.

CONCLUSIONS

Consistent with previous reports, the sensitivity of the RIADT used in this study was low, due to early administration of the test. Administration of an RIADT too early after symptom onset causes false negative results. In the influenza epidemic season, practitioners should not use RIADT for patients with upper respiratory symptoms and high fever for at least 12 hours after onset. A positive RIADT result after this gives the physician firm support for a diagnosis of influenza. A negative RIADT result does not mean 'no influenza'. Presence of high fever and chills might predict influenza, but additional tests are necessary for patients with specific symptoms inconsistent with a diagnosis of influenza virus infection.

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Contributors

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CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest regarding the publication of this research.

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STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	#1, 3
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	#7
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	#8
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	#8
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	#8
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	#8
Test methods	7	The reference standard and its rationale.	#10, 20
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	#9, 10
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	#9, 10
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	#9, 10
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	No
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	#11
	13	Methods for calculating test reproducibility, if done.	No
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	#11
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	#13,15,16
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	#8,11
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	#10
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	No
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	NA
	20	Any adverse events from performing the index tests or the reference standard.	No
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	#12,14
	22	How indeterminate results, missing data and outliers of the index tests were handled.	No
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	NA
	24	Estimates of test reproducibility, if done.	NA
DISCUSSION	25	Discuss the clinical applicability of the study findings.	#19,20