J Infect Chemother 25 (2019) 578–583

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Analytical and clinical evaluation of a point-of-care molecular diagnostic system and its influenza A/B assay for rapid molecular detection of the influenza virus☆

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ARTICLE INFO

Article history:
Received 22 November 2018
Received in revised form
28 January 2019
Accepted 26 February 2019
Available online 21 March 2019

Keywords:
Cobas Liat system
Influenza
Point-of-care testing
Cobas Influenza A/B

ABSTRACT

Recently, rapid molecular detection systems have been used for point-of-care testing for the diagnosis of influenza worldwide. Here, we evaluated the performance of the cobas Liat system and the cobas Influenza A/B assay (Liat) using fresh nasopharyngeal samples collected from a Japanese population between December 2017 and February 2018. The performance of the examination was compared with that of antigen testing and a conventional polymerase chain reaction (nested-PCR) method. A total of 159 patients were included in this study, and 77 tested positive using Liat. The concordance rate between Liat and nested PCR was 97.5%. The median time between the ordering of testing and completion of molecular analyses using Liat was 30 min (interquartile range: 28–35 min). The overall sensitivity and specificity of antigen testing were 57.1% and 100%, respectively. The duration from symptom onset to examination did not alter antigen testing sensitivity. The current study demonstrates the high performance of Liat for the rapid molecular identification of the influenza virus.

1. Introduction

The influenza virus is a leading causative pathogen of respiratory infections in winter. Although influenza is generally self-limited, the Centers for Disease Control and Prevention (CDC) in the United States recommend that patients at high risk receive early treatment with antiviral drugs [1]. Influenza is additionally a concern among healthcare workers and inpatients owing to the risk of its transmission in hospital settings [2]. The early and reliable diagnosis of influenza is crucial for effective treatment and infection control.

The point-of-care testing of influenza has become widely applicable since the introduction of antigen testing [3], although physicians often encounter false-negative results due to insufficient influenza viral load in patient samples [4]. Rapid molecular detection systems, therefore, have been developed and employed worldwide for point-of-care testing with high sensitivity [4]. In Japan, however, the only approved molecular detection system for the diagnosis of influenza is the Loopamp A influenza-virus detection kit (Eiken Chemical, Tokyo, Japan). This system has its limitations, in that it is only capable of detecting the influenza type A virus, and sample preparation must be performed on ice.

The cobas Liat system and the cobas Influenza A/B assay (Liat: Roche Molecular Systems, CA, U.S.), which are classified as small equipment, received a Clinical Laboratory Improvement

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https://doi.org/10.1016/j.jiac.2019.02.022
1341-321X/

Amendments waiver by the U.S. Food and Drug Administration in 2015 [5]. Several recent studies have reported their high analytical performance for the detection of the influenza virus [6,7]. The system requires only three simple steps and automatically performs real-time PCR to detect the influenza virus; the result is available within 20 min [5].

In the current study, we evaluated the clinical and analytical performance of Liat, comparing it with those of antigen testing and conventional PCR method.

2. Patients and methods

We conducted a prospective single-center observational study at Tsukuba Medical Center Hospital (TMCH: 453 beds). TMCH, an acute-care teaching hospital located in Tsukuba city in Japan, serves as a primary pediatric emergency center and tertiary emergency medical center. The study was carried out between 28 December 2017 and 2 February 2018. The ethical committee of TMCH approved the current study (approval number: 2017-055). Written informed consent was obtained from all participants in this study.

2.1. Inclusion criteria for patients

We enrolled all patients who were suspected of having influenza based on physician assessment and considered clinically eligible for influenza antigen testing. The majority of patients included had flu-like symptoms and were comprised of health-care workers, emergency department patients examined by researchers (HS or YA), and inpatients.

2.2. Data collection

For background data, we collected information on age, sex, comorbidities, influenza vaccination history, close contact with confirmed influenza patients, occupation, prior use of anti-influenza drugs, and the requirement for hospitalization. The following signs and symptoms were also recorded: fever (body temperature $\geq$37.8 °C), chill or subjective fever, cough, sputum production, fatigue, sore throat, myalgia or arthralgia, headache, runny nose or nasal obstruction, diarrhea, hypoxia, conjunctivitis, pharyngeal erythema, tonsillar swelling or exudate, cervical lymphadenopathy, and abnormal sound on chest auscultation.

2.3. Sample collection and examination for detection of influenza virus

Two nasopharyngeal samples were collected from each patient. A sample for antigen testing (RapidTesta color FLU stick, Sekisui Medical, Tokyo, Japan) was obtained with a swab supplied with the kit, and a sample for Liat was collected with a flocked swab and then suspended in a universal transporting medium (UTM Viral Transport Medium, Copan Diagnostics, CA, U.S.). We promptly transported the samples to a laboratory and performed antigen testing and nucleic acid amplification testing using Liat. Liat was placed in a biosafety level 2 microbiological laboratory in TMCH, and examiners wore protective equipment, such as masks and gowns, during the examination. Researchers (HS and YA) either collected samples from patients or directly checked the procedure used for sample collection by the treating physicians and nurses.

Results and estimated cycle threshold values (Ct) from sample analyses using Liat were determined by referring to stored files in the instrument and amplification plots displayed on its monitor. If results were erroneous, we re-analyzed the same sample using Liat. If both first and second Liat examinations showed an erroneous result and displayed an amplification plot for a positive reaction, samples were diluted, and a third examination was performed because this erroneous result may have been due to excess viral load. Residual samples in universal transporting media were stored in a freezer at −80 °C immediately after examinations using Liat. These frozen samples were collected weekly and sent to a centralized laboratory (SRL, Tokyo, Japan) for analysis by a conventional PCR assay (nested PCR [8]) for the detection of the influenza virus. We later examined the agreement of the results between Liat and nested PCR. A true positive for influenza virus was defined as a positive result from examination by either Liat or nested PCR, whereas a true negative was considered as negative results from examination by both Liat and nested PCR.

2.4. Statistical analyses

The sensitivity and specificity of antigen testing were calculated using the Clopper and Pearson method, with 95% confident intervals (95% CI). Categorical variables were compared by using the Fisher exact test, and p-values <0.05 were considered to represent statistically significant differences. We performed univariate and multivariate logistic regression analyses for calculating odds ratios (OR) and for identifying variables associated with influenza. Variables with p-values <0.05 in the univariate analysis were included in the multivariate logistic regression analysis after considering potentially confounding factors. All calculations were conducted using R 3.3.1 (The R Foundation, Vienna, Austria).

3. Results

3.1. Demographic data of all patients and analysis of patient samples using Liat

We examined 159 individual patients for the detection of the influenza virus using both antigen testing and Liat. The median number of Liat examinations per day was 3 (interquartile range [IQR]: 2–5), and the maximum number was 30 on 1st January 2018. The median time from ordering influenza examinations to obtaining the Liat results was 30 min (IQR: 28–35 min, Fig. 1a). Twelve patients (7.5%) underwent re-examination due to erroneous results. All 12 patients re-examined were positive for antigen testing (type A: 2 and type B: 10). In these patients, we observed amplification failure of an internal control or indeterminate results, which may have been due to high viral loads in samples (low Ct value was indicated), although the amplification plot showed a positive reaction. Re-examination of the samples using Liat provided a positive result in all 12 patients, of whom seven had re-examination using diluted samples.

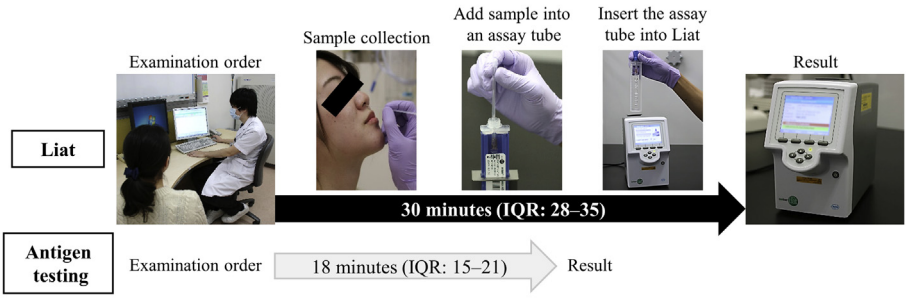
Table 1 describes the demographic data of all 159 patients, of whom 87 (54.7%) were female. The median age was 34 years, ranging from 9 months to 95 years, and 18 patients (11.3%) were over 65 years of age. Seventy-five patients (47.2%) were healthcare workers, and 24 (15.1%) patients were inpatients.

3.2. Comparison of results between Liat and nested PCR, and demographic data of patients for whom positive results were obtained by Liat

Of the 159 patients, 77 (48.4%) showed a positive result for Liat (type A: 36, type B: 41). Four patients had a discordant result between Liat and nested PCR; all 4 patients were Liat positive/nested-PCR negative and Ct >30.

Among 77 patients who were positive for Liat (female, 51.9%), 33 were healthcare workers (42.9%), 6 (7.8%) were inpatients (Table 1), 22 patients (28.6%) had a history of close contact with

a) Time from ordering an examination to obtaining the result



b) Comparison of the results and the sensitivity/specificity of antigen testing

Influenza A & B

		Liat	
		Pos	Neg
Antigen testing	Pos	44	0
	Neg	33	82

Sensitivity
57.1% (95% CI: 45.4–68.4)
Specificity
100% (95% CI: 95.6–100)

Influenza A

		Liat	
		Pos	Neg
Antigen testing	Pos	22	0
	Neg	14	82

Sensitivity
61.1% (95% CI: 43.5–76.9)
Specificity
100% (95% CI: 95.6–100)

Influenza B

		Liat	
		Pos	Neg
Antigen testing	Pos	22	0
	Neg	19	82

Sensitivity
53.7% (95% CI: 37.4–69.3)
Specificity
100% (95% CI: 95.6–100)

Fig. 1. Performance comparison between Liat and antigen testing.

Table 1
Demographic data for all patients.

	Overall	Positive results obtained by Liat		
		Total	Influenza A	Influenza B
	n = 159	n = 77	n = 36	n = 41
	(100%)	(48.4%)	(22.6%)	(25.8%)
Age (median [IQR])	34 [25, 45]	36 [23, 43]	36 [25, 42]	37 [23, 44]
<18 yrs	9 (5.7)	3 (3.9)	1 (2.8)	2 (4.9)
18–65 yrs	132 (83.0)	70 (90.9)	33 (91.7)	37 (90.2)
>65 yrs	18 (11.3)	4 (5.2)	2 (5.6)	2 (4.9)
Female	87 (54.7)	40 (51.9)	18 (50.0)	22 (53.7)
Close contact with influenza patients	31 (19.5)	22 (28.6)	12 (33.3)	10 (24.4)
Healthcare worker	75 (47.2)	33 (42.9)	14 (38.9)	19 (46.3)
Inpatient	24 (15.1)	6 (7.8)	2 (5.6)	4 (9.8)
Comorbidities	35 (22.0)	12 (15.6)	6 (16.7)	6 (14.6)
Immunosuppressive state	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Asthma	15 (9.4)	8 (10.4)	5 (13.9)	3 (7.3)
Chronic lung disease except for asthma	5 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
Neurological disease	5 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
Heart disease	4 (2.5)	2 (2.6)	0 (0.0)	2 (4.9)
Diabetes mellitus	4 (2.5)	1 (1.3)	1 (2.8)	0 (0.0)
Influenza vaccine immunization	92 (57.9)	37 (48.1)	15 (41.7)	22 (53.7)
Prior use of anti-influenza drugs	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Signs and symptoms				
Fever ≥37.8 °C	103 (64.8)	54 (70.1)	30 (83.3)	24 (58.5)
Chill or subjective fever	111 (69.8)	59 (76.6)	28 (77.8)	31 (75.6)
Cough	97 (61.0)	62 (80.5)	31 (86.1)	31 (75.6)
Sputum production	26 (16.4)	18 (23.4)	9 (25.0)	9 (22.0)
Fatigue[a]	83/151 (55.0)	48/75 (64.0)	21/35 (60.0)	27/40 (67.5)
Sore throat[a]	81/151 (53.6)	47/75 (62.7)	23/35 (65.7)	24/40 (60.0)
Myalgia or arthralgia[a]	84/151 (55.6)	50/75 (66.7)	26/35 (74.3)	24/40 (60.0)
Headache[a]	80/151 (53.0)	43/75 (57.3)	26/35 (74.3)	17/40 (42.5)
Runny nose or nasal obstruction	91 (57.2)	48 (62.3)	19 (52.8)	29 (70.7)
Diarrhea	7 (4.4)	3 (3.9)	1 (2.8)	2 (4.9)
Hypoxia	5 (3.1)	3 (3.9)	1 (2.8)	2 (4.9)
Conjunctivitis	3 (1.9)	2 (2.6)	2 (5.6)	0 (0.0)
Pharyngeal erythema	100 (62.9)	56 (72.7)	26 (72.2)	30 (73.2)
Tonsillar swelling or exudate	9 (5.7)	3 (3.9)	2 (5.6)	1 (2.4)
Cervical lymphadenopathy	7 (4.4)	2 (2.6)	1 (2.8)	1 (2.4)
Wheeze on chest auscultation	5 (3.1)	3 (3.9)	2 (5.6)	1 (2.4)

[a] Data were not available for infants and unconscious patients.

influenza patients, 37 (48.1%) had received the influenza vaccination, 54 (70.1%) showed fever, and 62 (80.5%) had a cough. Table 2 provides the odds ratios of each variable for predicting influenza. Multivariate logistic regression analysis revealed that close contact with influenza patients (OR: 4.13, 95% CI: 1.36–12.5), cough (OR: 8.36, 95% CI: 3.22–21.7), and fatigue (OR: 3.34, 95% CI: 1.34–8.35) were positively associated with influenza, whereas influenza vaccination was negatively associated (OR: 0.15, 95% CI: 0.05–0.40).

3.3. Impact of illness duration and Ct value on the diagnostic performance of antigen testing

The sensitivity of antigen testing was 57.1% (95% CI: 45.4–68.4) for influenza A and B, 61.1% (95% CI: 43.5–76.9) for influenza A, and 53.7% (95% CI: 37.4–69.3) for influenza B (Fig. 1b). We did not identify any false-positive results among antigen-positive patients. Table 3 demonstrates that the duration from the onset of any symptoms to influenza examination did not significantly influence the sensitivity of antigen testing ($p = 0.59$). Similarly, the sensitivity of antigen testing was unchanged over time when we limited the onset symptoms to body temperature ≥37 °C, chill, and subjective fever ($p = 0.43$).

Table 4 shows the change in the sensitivity of antigen testing according to the Ct value of Liat. The sensitivity of antigen testing in patients with Ct <20, ≥20 to <30, and ≥30 was 89.7% (95% CI: 75.8–97.1), 32.1% (95% CI: 15.9–52.4), and 0% (95% CI: 0–30.8), respectively.

4. Discussion

Ip et al. showed that the viral shedding load in symptomatic influenza patients was 5.4 $\log_{10}$ copies/mL on average, peaking on the day of symptom onset and decreasing thereafter [9]. Traditional antigen testing for influenza requires a viral load of at least 6 $\log_{10}$ copies/mL in a sample as a detection limit [10,11]. According to a recent systematic review, the sensitivities for detecting influenza type A/B were 54.4%/53.2% for traditional antigen testing, 80.0%/76.8% for digital immunoassays, and 91.6%/95.4% for rapid molecular detection systems [4]; however, the sensitivity of molecular detection systems varies depending on the equipment used [12].

Several studies have evaluated the analytical performance of Liat as a molecular detection system for the influenza virus. Nolte et al. examined the sensitivity of Liat and Alere i Influenza A & B (Abbott, IL, U.S.) using frozen-stored nasopharyngeal samples that had tested positive for the influenza virus using a different molecular detection system [6]. In their study, the Alere i Influenza A & B showed a positive rate of 71.3% for type A and 93.3% for type B influenza virus, whereas Liat successfully detected the influenza virus in all samples [6]. In another study, Young et al. evaluated the sensitivity and specificity of Liat using fresh nasopharyngeal samples, with data from a conventional RT-PCR testing as a reference [7]. The reported sensitivity and specificity of Liat were 100% and 98.3% for type A, and 94.4% and 100% for type B influenza viruses [7].

Our study demonstrates that in patients suspected of having influenza, the results of Liat showed high agreement with those of nested PCR in terms of the detection of both influenza type A and

Table 2
Odds ratios of variables associated with influenza.

	Crude odds ratio (95% CI)		*p*-value	Adjusted odds ratio (95% CI)		*p*-value
Age Category						
<18 yrs	Reference					
18–65 yrs	2.26	(0.20–25.5)	0.26			
>65 yrs	0.67	(0.05–9.47)	0.54			
Female	0.79	(0.41–1.50)	0.50			
Close contact with influenza patients	3.31	(1.36–8.04)*	0.007	4.13	(1.36–12.5)*	0.01
Healthcare worker	0.64	(0.33–1.21)	0.29			
Inpatient	0.30	(0.11–0.80)*	0.02	0.23	(0.05–1.02)	0.05
Comorbidities						
Immunosuppressive state	NA[a]		0.99			
Asthma	1.18	(0.40–3.43)	0.69			
Chronic lung disease except for asthma	NA[a]		0.99			
Neurological disease	NA[a]		0.99			
Heart disease	1.01	(0.14–7.39)	0.95			
Diabetes mellitus	0.50	(0.04–5.63)	0.36			
Influenza vaccine immunization	0.40	(0.20–0.78)*	0.02	0.15	(0.05–0.40)*	<0.001
Prior use of anti-influenza drugs	NA[a]		0.99			
Signs and symptoms						
Fever ≥37.8 °C	1.56	(0.80–3.05)	0.17			
Chill or subjective fever	1.85	(0.91–3.74)	0.07			
Cough	5.68	(2.72–11.9)*	<0.001	8.36	(3.22–21.7)*	<0.001
Sputum production	2.82	(1.15–6.94)*	0.02	1.16	(0.38–3.58)	0.79
Fatigue	2.08	(1.08–4.00)*	0.03	3.34	(1.34–8.35)*	0.01
Sore throat	2.07	(1.08–3.97)*	0.03	1.24	(0.54–2.82)	0.61
Myalgia or arthralgia	2.47	(1.28–4.78)*	0.007	1.14	(0.49–2.70)	0.76
Headache	1.42	(0.75–2.69)	0.29			
Runny nose or nasal obstruction	1.43	(0.75–2.73)	0.21			
Diarrhea	0.75	(0.16–3.47)	0.76			
Hypoxia	1.54	(0.25–9.50)	0.60			
Conjunctivitis	2.05	(0.18–23.2)	0.53			
Pharyngeal erythema	1.97	(1.00–3.89)*	0.01	1.42	(0.59–3.41)	0.44
Tonsillar swelling or exudate	0.49	(0.12–2.02)	0.36			
Cervical lymphadenopathy	0.39	(0.07–2.07)	0.30			
Wheeze on chest auscultation	2.05	(0.18–23.2)	0.60			

Odds ratios are calculated using logistic regression analysis.
*p-value<0.05.
[a] Odds ratios are unavailable because no influenza patients have these characteristics.

Table 3
Timing of examination and the sensitivities/specificities of antigen testing.

Duration of symptoms at the examination	Antigen testing			
	Sensitivity	*p*-value	Specificity	*p*-value
Any symptoms				
<12 h	50.0 (18.7–81.3)	0.59	100 (85.2–100)	1.0
12–24 h	50.0 (29.1–70.9)		100 (87.7–100)	
24–48 h	58.6 (38.9–76.5)		100 (81.5–100)	
>48 h	71.4 (41.9–91.6)		100 (75.3–100)	
Body temperature ≥ 37 °C, chill or subjective fever				
<12 h	51.9 (18.7–81.3)	0.43	100 (88.8–100)	1.0
12–24 h	72.7 (49.8–89.3)		100 (87.7–100)	
24–48 h	52.6 (28.9–75.6)		100 (73.5–100)	
>48 h	66.7 (9.40–99.2)		100 (47.8–100)	
Overall	57.1 (45.4–68.4)		100 (95.6–100)	

Sensitivities and specificities are provided with 95% confident interval.

Table 4
Antigen testing sensitivities stratified by the estimated cycle threshold (Ct) value of Liat.

	Antigen testing sensitivity		
	Total	Influenza A	Influenza B
Estimated Ct of Liat			
<20	89.7% (35/39)	84.2% (16/19)	95.0% (19/20)
≥20 to <30	32.1% (9/28)	42.9% (6/14)	21.4% (3/14)
≥30	0.0% (0/10)	0.0% (0/3)	0.0% (0/7)

type B viruses; this finding supports the high diagnostic performance of Liat. Further, results could be obtained rapidly using Liat, since the median time from ordering an examination to obtaining the first result was 30 min. This duration was comparable with that required for routine blood examination or plain radiography, which indicated a higher convenience and utility of Liat in daily practice compared to conventional molecular diagnostic tools. However, some drawbacks of Liat exist in comparison with antigen testing, i.e., the limitation on the number of examinations per day, high cost, and the requirement for re-examination in few cases as described in the current study.

The current guidelines of the Infectious Disease Society of America recommend that molecular examinations be used for the diagnosis of influenza in high-risk patients [13]. Patients aged ≥65 years or <2 years, pregnant women, or persons with medical conditions, immunosuppression, or morbid obesity are known to be at risk of developing serious complications [1]. Rapid and accurate diagnosis made by point-of-care molecular examinations can encourage physicians to initiate prompt antiviral treatment in high-risk patients, which may lead to improvements in mortality [14]. Healthcare workers and hospitalized patients are also eligible for molecular examination, considering that they can be a reservoir of influenza in healthcare settings [1,2]. Point-of-care molecular examinations may help in the early isolation of influenza-infected healthcare workers or inpatients and could reduce the risk of exposure to influenza in high-risk patients. In contrast, molecular examinations in previously healthy patients may be warranted only if the results are likely to affect antiviral treatment decisions or reduce inappropriate antibiotic prescription [1,13]. Further studies are necessary to demonstrate the necessity and clinical utility of rapid point-of-care molecular examinations because of the paucity of data in an actual clinical setting.

Antigen testing showed high specificity but low sensitivity in our study, as consistent with previous research [3]. We additionally investigated the factors that influence the sensitivity of antigen testing. A meta-analysis implied that the sensitivity of antigen testing is low on the first day of symptom onset [3]. However, we were unable to find data for the relationship between the sensitivity of antigen testing and illness duration (Table 3), and this result did not change even though two different definitions of symptom onset were applied. On the other hand, the viral load in samples seemed to dramatically affect the sensitivity of antigen testing. As shown in Table 4, the sensitivity of antigen testing decreased in line with the increase in the Ct value of Liat (decrease in viral load).

Several limitations associated with the present study warrant mention. First, four patients were Liat positive/nested PCR negative, and therefore showed discordant results. Liat revealed Ct ≥30 in these patients, suggesting that the viral load in the samples was low. The loss of virus during the frozen storage process of residual samples may have led to negative results of nested PCR. Second, we estimated Ct mainly from an amplification plot; consequently, the Ct values in our study may inaccurately reflect the viral load. Third, the sensitivity of antigen testing may have decreased because we did not use flocked swabs for sample collection. In addition, we used conventional antigen testing, which requires the visual interpretation of a result. Digital immunoassays, such as silver amplification immunochromatography system, are novel antigen testing methods that have shown higher sensitivity than conventional antigen testings [15]. The clinical utility of digital immunoassays should be studied in comparison with that of rapid molecular detection systems.

In conclusion, our study demonstrated that Liat enables rapid and highly accurate molecular detection of the influenza virus. Further studies should investigate the clinical utility of such rapid molecular detection systems in different clinical settings.

Conflicts of interest

Roche Diagnostics provided fees for research expenses and lent the cobas Liat system and the cobas Influenza A/B assay without charge. The funder had no involvement with study design, conduct and management, collection or interpretation of data, or preparation of the manuscript.

Acknowledgments

We thank Mr. Keiji Iida (SRL Inc.) for providing relevant information about molecular examinations and Mr. Akihito Nakamaru (SRL Inc.) for building the transport system of samples.

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