



Evaluation of a novel rapid TRC assay for the detection of influenza using nasopharyngeal swabs and gargle samples

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Abstract

We evaluated a novel transcription-reverse transcription concerted reaction (TRC) assay that can detect influenza A and B within 15 min using nasopharyngeal swab and gargle samples obtained from patients with influenza-like illness, between January and March 2018 and between January and March 2019. Based on the combined RT-PCR and sequencing results, in the nasal swabs, the sensitivity and specificity of TRC for detecting influenza were calculated as 1.000 and 1.000, respectively. In the gargle samples, the sensitivity and specificity of TRC were 0.946 and 1.000, respectively. The TRC assay showed comparable performance to RT-PCR in the detection of influenza viruses.

Keywords Rapid detection · Influenza · TRC method; RT-PCR

Introduction

The transcription-reverse transcription concerted reaction (TRC) method is a combination of direct and rapid isothermal RNA amplification and real-time identification using an intercalation-activating fluorescence (INAF)

probe [1, 2]. In Europe, Japan, and Vietnam, TRC ready-to use reagents have been used for the diagnosis of tuberculosis [3], nontuberculous mycobacterial infections, *Chlamydia* infection, gonorrhea, and mycoplasma pneumonia. Recently, a novel TRC assay that can detect influenza A and B within 15 min was developed [4, 5]. In this study, we evaluated the efficacy of the novel rapid TRC assay for detecting influenza viruses in nasopharyngeal swab and gargle samples obtained from patients with influenza-like illness.

All authors meet the ICMJE authorship criteria.

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Materials and methods

Ethics

This study was approved by the ethics committee of Nagasaki University Hospital (approval numbers: 17121822 and 18111919) and was registered at the UMIN Clinical Trials Registry (reference numbers: UMIN000032395 and UMIN000034545). Written informed consent for participation in and publication of this study was obtained from all participants before sample collection.

Study design

A prospective observational study was conducted in period 1, between January and March 2018, and period 2, between January and March 2019. Patients who visited or were hospitalized at the Department of Respiratory Medicine, Japanese Red Cross Nagasaki Genbaku Hospital, with influenza-like illness (ILI) [6] were included in this study. Patients were excluded if they were administered anti-influenza agents within 1 month prior to the day on which they were sampled. Nasopharyngeal swabs were collected from patients in both periods using two swabs: one was used for antigen testing using silver amplification immunochromatography (FUJI DRI-CHEM IMMUNO AG Cartridge FluAB, Fujifilm, Kanagawa, Japan) [7] at Nagasaki Genbaku Hospital and the other was stored at -20°C until further analysis. Gargle samples were collected from patients in period 2. For gargle sampling, the patients gargled their throat with 20 mL distilled water for 10 s. The physicians determined the clinical diagnosis with history taking, physical findings, and results of the influenza antigen test. All information, such as clinical report forms and results of the TRC and RT-PCR, was summarized and analyzed at Nagasaki University Hospital.

Sample analysis

We performed a rapid TRC assay based on the protocol described in Japan-patent (JP,2017-195871, A) at Nagasaki University Hospital. In summary, the procedure was performed as follows. A nasopharyngeal swab or gargle swab was mixed in 1-mL extraction buffer containing surfactant and incubated at 52°C for 1 min. Thirty microliters of the sample was mixed with dry reagent containing enzymes, substrates, primers, and INAF probes. The mixture was incubated at 46°C , and the fluorescence was monitored. RT-PCR and sequencing were performed as a gold standard at Tosoh Corporation based on the Influenza Diagnosis Manual [8–10]. Total RNA was isolated from 140 μL of TRC extraction buffer mixed with a nasopharyngeal swab or 140 μL of a gargle specimen using the Qiagen RNeasy kit. RT-PCR was performed using the One Step PrimeScript™ RT-PCR Kit (TAKARA BIO, Shiga, Japan). TRC assay and RT-PCR were performed independently, and the results of them were combined at Nagasaki University Hospital after all analysis was completed. If the results of the TRC and RT-PCR were different, the samples were analyzed by sequencing.

Statistical analysis

All statistical analyses were performed with EZR version 1.41 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [11], which is a graphical

user interface for R (the R Foundation for Statistical Computing, Vienna, Austria; version 3.6.3). Fisher's exact test was used to compare categorical variables, and the statistical significance level was set at <0.05 . The sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the TRC against the combined results of the RT-PCR and sequencing were calculated with 95% confidence intervals (95% CI).

Results

Patient characteristics

During the study period, a total of 188 patients were evaluated, comprising 92 patients in period 1 and 96 patients in period 2. Patient characteristics are shown in Table 1. Of the patients, 95 (50.5%) visited a hospital within 24 h of the onset of symptoms. The percentage of patients with fatigue was significantly lower in period 1 (37.0%) than in period 2 (71.9%, $P = <0.001$). The influenza antigen test using silver amplification immunochromatography detected influenza A and B in 38 (20.2%) and 39 (20.7%) patients, respectively. The percentage of influenza A was significantly lower, while that of influenza B was significantly higher, in period 1 than in period 2 (Table 1).

Comparison of TRC and RT-PCR results

The results of the RT-PCR and TRC are shown in Table 2. In the nasal swabs, influenza A and B were detected using RT-PCR in 36 (19.1%) and 39 (20.7%) patients, respectively, and were detected using TRC in 38 (20.2%) and 40 (21.3%) patients, respectively (Table 2). Of all patients, three tested negative for influenza with RT-PCR, but positive with TRC. Influenza A was detected in two of these three patients, and influenza B was detected in one by sequencing. Based on the combined RT-PCR and sequencing results, the Se, Sp, PPV, and NPV of the TRC were 1.000, 0.973, 0.962, and 1.000, respectively (Table 3).

In the gargle samples, influenza A was detected by RT-PCR and TRC in 37 (38.5%) and 35 (36.5%) patients, respectively (Table 2). Of all patients in period 2, two tested positive for influenza A with RT-PCR, but negative with TRC. Influenza A was detected in these two patients by sequence analysis. Based on the combined RT-PCR and sequence analysis results, the Se, Sp, PPV, and NPV of the TRC in the gargle samples were 0.946, 1.000, 1.000, and 0.967, respectively (Table 3).

Table 1 Patient characteristics

Characteristic	Overall (N=188)		Period 1 (N=92)		Period 2 (N=96)		P value
	N	(%)	N	(%)	N	(%)	
Age (average ± S.D)	50.5	± 19.4	51.3	± 19.5	49.6	±19.5	NS
Gender = female	103	(54.8%)	58	(63.0%)	45	(46.9%)	0.029
Underlying diseases	94	(50.0%)	48	(52.2%)	46	(47.9%)	NS
Time since onset of symptoms							
0–12 h	48	(25.5%)	23	(25.0%)	25	(26.0%)	NS
12–24 h	47	(25.0%)	23	(25.0%)	24	(25.0%)	NS
24–48 h	39	(20.7%)	17	(18.5%)	22	(22.9%)	NS
48–72 h	19	(10.1%)	7	(7.6%)	12	(12.5%)	NS
72+ h	26	(13.8%)	14	(15.2%)	12	(12.5%)	NS
Unknown	9	(4.8%)	8	(8.7%)	1	(1.0%)	0.017
Symptoms							
Fever	143	(76.1%)	66	(71.7%)	77	(80.2%)	NS
Cough	124	(66.0%)	60	(65.2%)	64	(66.7%)	NS
Fatigue	103	(54.8%)	34	(37.0%)	69	(71.9%)	<0.001
Sore throat	100	(53.2%)	46	(50.0%)	54	(56.3%)	NS
Nasal discharge	99	(52.7%)	47	(51.1%)	52	(54.2%)	NS
Headache	70	(37.2%)	35	(38.0%)	35	(36.5%)	NS
Arthralgia	56	(29.8%)	23	(25.0%)	33	(34.4%)	NS
Myalgia	56	(29.8%)	22	(23.9%)	34	(35.4%)	NS
Diarrhea	11	(5.9%)	3	(3.3%)	8	(8.3%)	NS
Nausea	9	(4.8%)	3	(3.3%)	6	(6.3%)	NS
Results of influenza antigen test using silver amplification immunochromatography							
Influenza A	38	(20.2%)	3	(3.3%)	35	(36.5%)	<0.001
Influenza B	39	(20.7%)	38	(41.3%)	1	(1.0%)	<0.001
Negative	111	(59.0%)	50	(54.3%)	60	(62.5%)	NS

S.D. standard deviation, NS not significant

Comparison of results between nasopharyngeal swabs and gargle samples

In the RT-PCR testing, five patients tested negative for influenza A in their nasopharyngeal swabs, but tested positive for influenza A in their gargle samples (Fig. 1). The Se, Sp, PPV, and NPV of the RT-PCR in the gargle samples were 1.000, 0.922, 0.865, and 1.000, respectively. The Se, Sp, PPV, and NPV of the TRC in the gargle samples were 0.912, 0.935, 0.886, and 0.951, respectively.

Discussion

The novel rapid TRC assay showed great sensitivity and specificity in nasopharyngeal swabbing in both periods 1 and 2. Based on the results of the antigen test, period 1 was considered to be the influenza B epidemic season and period 2 was considered the influenza A epidemic season (Table 1). The sensitivity and specificity of the rapid TRC assay were 1.000

and 1.000, respectively, based on the combined RT-PCR and sequencing results for both periods. There are several rapid RT-PCR assays for detection of influenza, such as the ID Now influenza A and B 2 assay (ID Now), Cobas influenza A/B nucleic acid test (Liat), and Xpert Xpress Flu assay (Xpert). The previous studies reported that the sensitivity and specificity of these methods for detecting influenza A/B were 0.932 to 1.000/0.917 to 1.000 and 0.977 to 1.000/0.976 to 0.998, respectively [12–14]. These results indicate that the performance of the rapid TRC assay is comparable to that of rapid RT-PCR assays.

In the present study, the rapid TRC assay also showed great sensitivity and specificity for gargle sampling. The rapid TRC assay and RT-PCR detected influenza in more patients from gargle samples than from nasopharyngeal swabs. The sensitivity and specificity of the Rapid TRC assay were 0.946 and 1.000, respectively. The previous studies reported that the sensitivity of Xpert and Liat for gargle sampling was 0.917 and 1.000, respectively, in comparison with in-house RT-PCR [15, 16]. Although there are no data on the

Table 2 Results of RT-PCR and TRC

Item	Overall (N=188)		Period 1 (N=92)		Period 2 (N=96)		P value
	N	(%)	N	(%)	N	(%)	
RT-PCR in nasopharyngeal swab							
Influenza A	36	(19.1%)	4	(4.3%)	32	(33.3%)	<0.001
Subtype H1	9	(4.8%)	0	(0.0%)	9	(9.4%)	0.003
Subtype H3	27	(14.4%)	4	(4.3%)	23	(24.0%)	0.001
Influenza B	39	(20.7%)	39	(42.4%)	0	(0.0%)	<0.001
Negative	113	(60.1%)	49	(53.3%)	64	(66.7%)	NS
TRC in nasopharyngeal swab							
Influenza A	38	(20.2%)	4	(4.3%)	34	(35.4%)	<0.001
Influenza B	40	(21.3%)	40	(43.5%)	0	(0.0%)	<0.001
Negative	110	(58.5%)	48	(52.2%)	62	(64.6%)	NS
RT-PCR in gargle sample							
Influenza A	N/A		NCW		37	(38.5%)	N/A
Subtype H1	N/A		NCW		11	(11.5%)	N/A
Subtype H3	N/A		NCW		26	(27.1%)	N/A
Influenza B	N/A		NCW		0	(0.0%)	N/A
Negative	N/A		NCW		59	(61.5%)	N/A
TRC in gargle sample							
Influenza A	N/A		NCW		35	(36.5%)	N/A
Influenza B	N/A		NCW		0	(0.0%)	N/A
Negative	N/A		NCW		61	(63.5%)	N/A

NS not significant, N/A not applicable, NCW not complied with

Table 3 Performance of TRC assay for detection of influenza

	Overall	Period 1	Period 2
Nasopharyngeal swab			
TP	78	44	34
TN	110	48	62
FP	0	0	0
FN	0	0	0
Se (95% CI)	1.000 (0.931–1.000)	1.000 (0.882–1.000)	1.000 (0.851–1.000)
Sp (95% CI)	1.000 (0.951–1.000)	1.000 (0.891–1.000)	1.000 (0.915–1.000)
PPV (95% CI)	1.000 (0.931–1.000)	1.000 (0.882–1.000)	1.000 (0.851–1.000)
NPV (95% CI)	1.000 (0.951–1.000)	1.000 (0.891–1.000)	1.000 (0.915–1.000)
Gargle samples			
TP	35	N/A	35
TN	59	N/A	59
FP	0	N/A	0
FN	2	N/A	2
Se (95% CI)	0.946 (0.818–0.993)	N/A	0.946 (0.818–0.993)
Sp (95% CI)	1.000 (0.911–1.000)	N/A	1.000 (0.911–1.000)
PPV (95% CI)	1.000 (0.855–1.000)	N/A	1.000 (0.855–1.000)
NPV (95% CI)	0.967 (0.887–0.996)	N/A	0.967 (0.887–0.996)

Period 1 between January 1 and March 31, 2018; period 2 between January 1 and March 31, 2019; TP true positive; TN true negative; FP false positive; FN false negative; CI confidence interval; N/A not applicable

RT-PCR				TRC			
Nasopharyngeal swab				Nasopharyngeal swab			
		Pos	Neg			Pos	Neg
Gargle	Pos	32	5	Gargle	Pos	31	4
	Neg	0	59		Neg	3	58

Se, Sp, PPV, and NPV in gargle samples					
Se	1.000	(0.842-1.000)	0.912	(0.763-0.981)	
Sp	0.922	(0.827-0.974)	0.935	(0.843-0.982)	
PPV	0.865	(0.712-0.955)	0.886	(0.733-0.968)	
NPV	1.000	(0.911-1.000)	0.951	(0.863-0.990)	

Fig. 1 Comparison of gargle sample and nasopharyngeal swab results. The results obtained from gargle samples and nasopharyngeal swabs during period 2 were compared. Period 2 was between January 1 and March 31, 2019

specificity of rapid RT-PCR assays, the rapid TRC assay seems to be comparable with rapid RT-PCR assays for gargle samples. In the diagnosis of influenza, nasopharyngeal swabbing is the major sampling type, but these samples are difficult to obtain and the procedure is uncomfortable for patients [17]. In addition, due to the novel coronavirus (SARS-CoV-2) pandemic, healthcare workers must wear personal protective equipment when they obtain nasopharyngeal swabs. Therefore, it is vital to develop a diagnostic method, such as the rapid TRC assay, using gargle samples, that is easy to perform, non-invasive, material saving, and safe for healthcare workers [18].

There are some limitations in this study. First, the samples were obtained from one community hospital in Nagasaki, which might have limited the generalizability of the findings. Second, we used an equipment of the novel TRC assay under development. Accordingly, we are conducting a multicenter study for the rapid TRC assay using production version. Third, the rapid TRC assay was not compared with other rapid RT-PCR assays. Since these assays have not yet been approved by the Japanese Pharmaceuticals and Medical Devices Agency, we will conduct a comparative study in the future.

In conclusion, the novel rapid TRC assay showed comparable performance to RT-PCR in the detection of influenza viruses. In addition, because it could detect influenza viruses using gargle samples, the rapid TRC assay could contribute to the diagnosis of influenza during the SARS-CoV-2 pandemic.

Author contributions All authors meet the ICMJE authorship criteria.

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Availability of data and materials Raw data were generated at Nagasaki University Hospital. Derived data supporting the findings of this study are available from the corresponding author on request.

Declarations

Ethical approval This study was approved by the ethics committee of Nagasaki University Hospital (approval numbers: 17121822 and 18111919)

Consent to participate Written informed consent for participation in and publication of this study was obtained from all participants before sample collection.

Consent for publication Written informed consent for publication of this study was obtained from all participants before sample collection.

Competing interests The authors declare that they have no conflict of interest.

Disclaimer The sponsor had no control over the interpretation, writing, or publication of this work.

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