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RESEARCH ARTICLE

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Development of real-time fluorescent reverse transcription loop-mediated isothermal amplification assays for rhinovirus detection

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Abstract

Human rhinoviruses (RVs) belong to the genus *Enterovirus* of the family Picornaviridae, and are classified into RV-A, -B, and -C species. Two assays were developed to detect RVs by a real-time fluorescent reverse transcription loop-mediated isothermal amplification method: one was designed based on the 5′-untranslated regions (UTRs) of RV-A and -B, and the other was designed based on the 5′-UTR of RV-C. The competence of both assays for the diagnosis of RV infection was tested using isolated viruses and compared with real-time reverse transcription polymerase chain reaction assays on clinical specimens. Neither assay demonstrated cross-reactivity with other tested enteroviruses, and they detected 19 out of 21 tested RV-As and seven out of eight tested RV-Cs. The specificity of the assays was 100% for the detection of RVs and their sensitivity for RV-A and RV-C was 86.3% and 77.3%, respectively, on clinical specimens by the combined use of both assays. Considering that both developed assays were highly specific and detected the majority of recently circulating RVs, they are helpful for the diagnosis of RV infection. Consequently, the results generated by these assays will enhance the surveillance of respiratory illness and the study of the roles of RVs associated with clinical features and disease severity.

KEYWORDS
fluorescence, quenching primer, reverse transcription loop-mediated isothermal amplification, rhinovirus

1 | INTRODUCTION

Human rhinoviruses (RVs) are single-stranded, positive-RNA viruses belonging to the genus *Enterovirus* of the family Picornaviridae. The first RV was discovered in the 1950s, and approximately 100 RV serotypes were classified as RV-A or -B species by the early 1990s.[1-4] With the development of molecular diagnostic techniques, the RV-C group was identified in 2006.[5,6] The RV genome consists of structural (VP4, VP2, VP3, and VP1) and nonstructural (2A, 2B, 2C, 3A, 3B, 3C, and 3D) regions flanked by 5′- and 3′-untranslated regions (UTRs). Recently, a genotypic classification of RVs was proposed according to the sequence of VP1 or VP4/VP2, and more than 160 RVs have now been classified as RV-A, -B, or -C species (http://www.picornaviridae.com/).[7]

RVs cause respiratory illness throughout the world and throughout the year.[8] The studies using molecular methods and viral culture demonstrated that RVs are the most common cause of upper respiratory tract infections.[9] While they were once thought to cause relatively mild upper respiratory tract illness, RVs are suggested to be linked to exacerbations of the chronic pulmonary disease,

including asthma,[10] and chronic obstructive pulmonary disease.[11] RVs are reportedly detected in infants and children with severe bronchiolitis[12] as well as elderly patients with fatal pneumonia.[13]

Recently, nucleic acid amplification techniques have become major tools for the diagnosis of viral infections. Many real-time reverse transcription polymerase chain reaction (RT-PCR) assays for detecting RVs have been described to generate sensitive and quantitative results as laboratory diagnostic methods[14,15]; however, real-time RT-PCR methods require multiple steps, highly specialized technical skills, and take time to generate results (>1 hour). Loop-mediated isothermal amplification (LAMP), first reported in the early 2000s, is an isothermal nucleic acid amplification method, and a real-time LAMP (RT-LAMP) assay can be performed simply without the need for high-precision instruments and can detect viral genomes within 30 minutes.[16-18] A LAMP assay can be performed using fluorescent dyes for real-time monitoring or for judgment by the naked eye,[19,20] while fluorescent dyes have a major limitation in that they can bind nonspecifically to double-stranded DNA, such as primer-dimers, leading to erroneous results.[21] Previously, real-time fluorescent RT-LAMP was established to detect viral RNA using a quenching primer (QPrimer) or a quenching probe (QProbe). By using a QPrimer or QProbe, nonspecific reactions can be decreased and the target can be detected in a real-time manner.[22,23] In this study, two assays were developed to detect RVs by a real-time fluorescent RT-LAMP method, and the competence of both assays was compared with real-time RT-PCR assays for the detection of RV infection using clinical specimens.

2 | MATERIALS AND METHODS

2.1 | Clinical specimens and virus isolates

The viruses shown in Tables 2 and 3 were isolated from clinical specimens. RV-B14 (VR-284), human coxsackievirus B4 (VR-184) and B5 (VR-185), human enterovirus A71 (VR-1432), and enterovirus D68 (VR-1826) were obtained from the American Type Culture Collection (Manassas, VA). Nasopharyngeal swabs and nasal aspirates of patients enrolled in clinical studies approved by the ethics committees of the National Institute of Infectious Diseases, Showa General Hospital, Ishimemorial Aizenen, and Nakano Children's Hospital and screened positive for RV and/or other respiratory viruses by real-time RT-PCR[24-26] used for this study. This study was performed in compliance with the Declaration of Helsinki. Informed consent was obtained from all patients. A total of 132 nasopharyngeal swabs and nasal aspirates, consisting of 102, 2, 2, 2, 2, 2, 2, 1, 1, and 1 samples positive for RV, influenza A subtype H1pdm09 virus, influenza A subtype H3 virus, influenza B virus, respiratory syncytial virus A, respiratory syncytial virus B, human metapneumovirus, human parainfluenza virus type 3, human parainfluenza virus type 4, and human coronavirus OC43, respectively, and 15 samples negative for any virus tested were used.

2.2 | RNA preparation

Total RNA was prepared from the clinical specimens and isolated viruses using a MagMAX 96 Viral Isolation Kit (Thermo Fisher Scientific, Waltham, MA) (using 50 μL of the clinical specimens and isolated viruses and eluted in 50 μL) with KingFisher Flex (Thermo Fisher Scientific) according to the manufacturer's instructions.

2.3 | Sequencing of 5′-UTR region

Purified viral RNA from clinical specimens was reverse-transcribed using random hexamer primers (Promega, Madison, WI) and SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) according to the manufacturer's instructions. The complementary DNA (3 μL) was amplified in three separate PCRs using RV species A-, B-, and C-specific primer sets (0.5 μM of each, Table 1) with Phusion High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA). The following PCR cycling conditions were used: 98°C for 30 seconds, followed by 40 cycles of 98°C for 10 seconds, 65°C for 30 seconds, and 72°C for 1 minute, with a final extension of 72°C for 10 minutes. Approximately 600 bp amplified products were gel purified with a MinElute Gel Extraction Kit (Qiagen, Hilden, Germany), and sequencing was performed in both directions using the amplification primers and an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1 (Thermo Fisher Scientific).

2.4 | Preparation of RNA transcript control

To construct an RNA control to quantify RNA copy number by real-time RT-PCR, the 5′-UTR region of RV-A16 was amplified by RT-PCR, and the resulting PCR product containing the T7 promoter was transcribed in vitro. The detailed procedure is described below. The 5′-UTR region was amplified by PCR using Phusion High-Fidelity DNA Polymerase (New England Biolabs) with paired primers (TAATACGACTCACTATAGGGGTACWCTRKTAYTMYGGTAMYYTTGTACGCC and AGWGCATCKGGYAAYTTCCA; underlined sequence is T7 promoter region). RNA was transcribed using the T7 RiboMAX Express Large Scale RNA Production System (Promega) and treated with TURBO DNase (Thermo Fisher Scientific) to degrade template DNA. The dNTPs and NTPs were removed using MicroSpin G-25 Columns (GE Healthcare, Piscataway, NJ) by following the manufacturer's instructions. The transcribed RNAs were quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and RNA concentration was used to calculate the copy number of the transcribed RNA based on molecular weight and Avogadro's constant. The integrity of the transcribed RNAs was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

2.5 | Real-time RT-PCR assay

Real-time RT-PCR for quantifying virus genomes was carried out with a LightCycler 480 (Roche, Basel, Switzerland) as described previously for RVs[24] and other enteroviruses[14] with a slight modification using RNA transcript controls. Briefly, the 25-μL assay contained 12.5 μL of 2×

TABLE 1 Primer set

Names	Sequences (5′-3′)	Concentrations in RT-LAMP reactions, μM
Primers for RT-LAMP Set 1		
HRVA F3v1	CYAGCCTGCGTGGCTGCC	0.2
RhinoB3 1_1	GAAACACGGACACCCAAA	0.2
HRVA FIPv3	CATTCAGGGGCCGGAGGA-GACAAGGTGTGAAGAGYC	1.6
HRVA BIPv3	GGCTAACCTTAAMCCYGCAGC-GTAGTYGGTCCCATCCC	1.6
HRVA LFv1-1	CAAAACAAGCACACGG	0.38 + 0.02[a]
HRVA LFv2	CAAGATGAGCACACGC	0.38 + 0.02[a]
HRVA LBv4	ACAATCCAGTGTGTAGCTGGTCGTAA	0.8
Primers for RT-LAMP Set 2		
HRVC F3_1	AGCCTGCGTGGCTGCC	0.07
HRVC F3_2	TGCCTGCGTGGCTGCC	0.07
HRVC F3_3	AGCCCGCGTGGTGCCC	0.07
HRVC B3_2	GAAACACGGACACCCAAA	0.2
HRVC FIPv1_2	CATTCAGGGGCCGGAGGA-GACAGGGTGTGAAGGTTC	0.53
HRVC FIPv1_4	CATTCAGGGGCCGGAGGA-GACAGGGTGTGAAGATTC	0.53
HRVC FIPv1_7	CATTCAGGGGCCGGAGGA-GACAAGGTGTGAAGAGCC	0.53
HRVC BIPv1_5	GGCTAACCTTAACCCCGTAGC-GTAGTCGGTCCCATCCC	0.53
HRVC BIPv1_6	GGCTAATCCTAACCCCGTAGC-GTAGTTGGTTCCATCCC	0.53
HRVC BIPv1_7	GGCTAATCCAACCCYRCAGC-GTAGTCGGTTCCGTCCC	0.53
HRVC LFv3_1	CTCACWHGTAGCACAC	0.4
HRVC LFv3_2	CTCATADBDAGCACAC	0.4
HRVC LBv4_1	CCACCATGTAGGTAGTCGTAATGGGCAA	0.152 + 0.008[b]
HRVC LBv4_2	CCAGTGTGTATATAGTCGTAATGAGCAA	0.152 + 0.008[b]
HRVC LBv4_3	CCAGCATAAACACAGTCGTAATGGGCAA	0.152 + 0.008[b]
HRVC LBv4_4	CCAGTACAAACATGGTCGTAATGGGCAA	0.152 + 0.008[b]
HRVC LBv4_5	CCACCATGTAGATGGTCGTAATGAGCAA	0.152 + 0.008[b]
Primers for sequencing 5′-UTR		
HRVA seqF	GTATWCTRTTATTMCGGTAAAYTTGTACGCCA	
HRVA seqR	ACATTYTGTCTRGATACYTGWGCGCCCATG	
HRVB seqF	GTACACTGGTAYTWTGTACCTTTGTACGCCT	
HRVB seqR	GTGTWGAMACYTGWGCRCCCATGATYACA	
HRVC seqF	GTGCTCTTGTATYHCGGTACAYTTSCAYRCCA	
HRVC seqR	GYYTRSTWNACYTGDGCGCCCATGGTRACAA	

Abbreviations: RT-LAMP, reverse transcription loop-mediated isothermal amplification; UTR, untranslated region.
[a]Five percent of the LF primers were substituted for LF and LB.–>with QPrimer-5G for RT-LAMP Set 1 as described in Section 2.6.
[b]Five percent of the LB primers were substituted with QPrimer-5G for RT-LAMP Set 2 as described in Section 2.6.

RT-PCR buffer, 1 μL of 25× RT-PCR Enzyme Mix, 3.5 μL Primers/Probe Mix, 3 μL water, and 5 μL template RNA using AgPath-ID One-Step RT-PCR Reagents (Thermo Fisher Scientific). The primers/probe mix was prepared to contain 0.6 μM of each primer and 0.1 μM probe.

2.6 | Real-time fluorescent RT-LAMP assay

RT-LAMP was carried out as described previously with a slight modification,[22] as the 25-μL assay contained enzyme, buffer, primers, and template RNA. The primers were prepared to contain 0.2 μM each of the F3 and B3 primers, 1.6 μM each of the FIP and BIP primers, and 0.8 μM each of the LF and LB primers, as the final concentration in each reaction. Five percent of one of the LB or LF primers of each assay was substituted with QPrimer-5G (Nippon Steel and Sumikin Eco-Tech, Tokyo, Japan). The real-time RT-LAMP reaction was performed at 63°C for 30 minutes using a LightCycler 480 (Roche).

TABLE 2 The sensitivity of the RT-LAMP assays was tested using serial dilutions of viral RNA from several RV-A species.

		Numbers of positive replicates/number of tests for each assay, viral RNA concentration (copies/reactions)[a]						
		Set 1				Set 2		
Types	Virus isolate names	10^4	10^3	10^2	10^1	10^4	10^3	10^2
A10	0441-OsakaC-JPN-2015	NT	3/3	0/3	0/3	0/3	0/3	0/3
A12	25-SGH-JPN-2016	0/3	0/3	0/3	0/3	3/3	1/3	0/3
A16	0035-OsakaC-JPN-2015	NT	3/3	3/3	0/3	3/3	0/3	0/3
A18	4-SGH-JPN-2016	NT	3/3	2/3	0/3	0/3	0/3	0/3
A19	54-SGH-JPN-2015	3/3	1/3	0/3	0/3	0/3	0/3	0/3
A21	0063-OsakaC-JPN-2016	NT	3/3	2/3	0/3	0/3	0/3	0/3
A24	09-SGH-JPN-2015	NT	3/3	0/3	0/3	0/3	0/3	0/3
A28	0088-OsakaC-JPN-2016	NT	3/3	3/3	1/3	0/3	0/3	0/3
A34	0049-OsakaC-JPN-2015	NT	3/3	3/3	0/3	0/3	0/3	0/3
A40	0071-OsakaC-JPN-2016	NT	3/3	3/3	0/3	2/3	0/3	0/3
A45	0157-OsakaC-JPN-2016	0/3	0/3	0/3	0/3	0/3	0/3	0/3
A46	29-SGH-JPN-2015	NT	3/3	1/3	1/3	0/3	0/3	0/3
A49	0044-OsakaC-JPN-2015	NT	NT	3/3	0/3	NT	0/3	0/3
A54	0058-OsakaC-JPN-2015	NT	0/3	0/3	0/3	3/3	0/3	0/3
A58	0008-OsakaC-JPN-2015	NT	3/3	1/3	0/3	3/3	0/3	0/3
A59	0087-OsakaC-JPN-2016	NT	3/3	3/3	3/3	0/3	0/3	0/3
A75	0123-OsakaC-JPN-2016	NT	3/3	3/3	1/3	3/3	0/3	0/3
A81	0073-OsakaC-JPN-2016	NT	3/3	3/3	1/3	0/3	0/3	0/3
A82	54-SGH-JPN-2014	NT	3/3	1/3	0/3	0/3	0/3	0/3
A88	12-SGH-JPN-2015	NT	3/3	1/3	0/3	NT	0/3	0/3
A101	41-SGH-JPN-2015	0/3	0/3	0/3	0/3	1/3	0/3	0/3
B14	ATCC VR-284	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Abbreviations: NT, not tested; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RV, rhinovirus.
[a]Viral RNA copy number was calculated based on the 5′-untranslated regions of each viral gene as described in the text.

TABLE 3 The sensitivity of the RT-LAMP assays was tested using serial dilutions of viral RNA from several RV-C species

		Number of positive replicates/number of tests for each assayviral RNA concentration (copies/reactions)[a]						
		Set 1			Set 2			
Types	Virus isolate names	10^5	10^4	10^3	10^5	10^4	10^3	10^2
C02	0095-OsakaC-JPN-2016	1/3	0/3	0/3	NT	3/3	0/3	0/3
C06	0060-OsakaC-JPN-2016	0/3	0/3	0/3	NT	3/3	3/3	0/3
C09	47-SGH-JPN-2015	0/3	0/3	0/3	NT	3/3	3/3	1/3
C12	0153-OsakaC-JPN-2016	1/3	0/3	0/3	NT	3/3	3/3	0/3
C18	57-SGH-JPN-2014	0/3	0/3	0/3	NT	3/3	1/3	0/3
C23	0105-OsakaC-JPN-2016	0/3	0/3	0/3	0/3	0/3	NT	NT
C40	63-SGH-JPN-2016	NT	0/3	0/3	3/3	1/3	0/3	NT
C53	04-SGH-JPN-2015	0/3	0/3	0/3	NT	3/3	3/3	2/3

Abbreviations: NT, not tested; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RV, rhinovirus.
[a]Viral RNA copy number was calculated based on the 5′-untranslated regions of each viral gene as described in the text.

TABLE 4 The performance of the RT-LAMP assays compared with a real-time RT-PCR assay on RV-A-positive specimens

Real-time RT-PCR	RT-LAMP		Specificity; 95% CI	Sensitivity; 95% CI
	Positive	Negative		
Positive	63	10	100.0%;88.4%-100.0%	86.3%;76.3%-93.2%
Negative	0	30		

Abbreviations: CI, confidence interval; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; RV, rhinovirus.

3 | RESULTS

3.1 | Development of the RT-LAMP assays

To design primer sets for RT-LAMP, the 5′-UTR regions of RVs were sequenced using 102 clinical specimens that were positive for RV by real-time RT-PCR. The 5′-UTR sequences of 76 RV-A, 4 RV-B, and 26 RV-C specimens were obtained and deposited at the National Center for Biotechnology Information (accession numbers, LC420517-LC420622). Two 5′-UTR sequences in the same specimen were obtained from four samples; the one for RV-A and the other for RV-C were obtained from three samples, while the one for RV-B and the other for RV-C were obtained from one specimen. Primers for the RT-LAMP Set 1 assay were designed based on 76 RV-A and four RV-B sequences, while those for the RT-LAMP Set 2 assay were designed according to 26 RV-C sequences (Table 1). The specificity of each RT-LAMP assay was evaluated using other enteroviruses (human coxsackievirus B4 and B5, human enterovirus A71, and enterovirus D68), and no cross-reactivity was detected (data not shown).

The sensitivity of each RT-LAMP assay was evaluated using serially diluted RNAs that were purified from several clinical isolates of RV-A, -B, and -C (Tables 2 and 3). The assays were carried out independently three times. The majority of tested RVs were detected by either or both of the RT-LAMP assays, but their respective sensitivity for each RV was different (Tables 2 and 3). Both RT-LAMP assays barely reacted with RV-A45, A101, B14, and C23 (Tables 2 and 3). The RT-LAMP Set 1 assay detected at least 1.0×10^2, 10^3, and 10^4 copies/reaction of RV-A16, A28, A34, A40, A49, A59, A75, and A81, RV-A10, A18, A21, A24, A46, A58, A82, and A88, and RV-A19 in all three tests, respectively (Tables 2 and 3). The RT-LAMP Set 2 assay detected at least 1.0×10^3, 10^4, and 10^5 copies of RV-C06, C09, C12, and C53, RV-A12, A16, A54, A58, A75, C02, and C18, and RV-C40 in all three tests, respectively (Tables 2 and 3). Representative examples of the quenching signals of RT-LAMP Set 1 on RV-As and those of RT-LAMP Set 2 on RV-Cs were shown in Figure S1. The RT-LAMP Set 1 assay reacted with some RV-Cs and with the majority of RV-As, while the RT-LAMP Set 2 assay reacted with some RV-As and with the majority of RV-Cs.

3.2 | Evaluation of the RT-LAMP assays using clinical specimens

The RT-LAMP assays were evaluated using RNA purified from 128 clinical specimens. Of them, 98 clinical specimens were positive for RV by real-time PCR and considered to be a single infection case with either RV-A, -B, or -C depending on the sequence of the 5′-UTR region, as described in Section 3.1. When either or both RT-LAMP assays detected the target, the results were taken as "positive." Neither RT-LAMP assay cross-reacted with 15 clinical specimens that were positive for influenza A subtype H1pdm09 virus, influenza A subtype H3 virus, influenza B virus, respiratory syncytial virus A or B, human metapneumovirus, human parainfluenza virus type 3 or 4, or human coronavirus OC43 or with 15 clinical specimens that were negative for all tested viruses. Consequently, it was shown that the specificity of the assays was 100% for detecting RVs (Tables 4 and 5). The assay results for 73 clinical specimens that were positive for RV-A by real-time RT-PCR and sequencing showed that the sensitivity of the RT-LAMP assays for RV-A was 86.3% (Table 4). Similarly, the results for 22 clinical specimens that were positive for RV-C by real-time RT-PCR and sequencing showed that the sensitivity of the RT-LAMP assays for RV-C was 77.3% (Table 5). Neither assay reacted with three clinical specimens that were positive for RV-B by real-time RT-PCR and sequencing (data not shown).

4 | DISCUSSION

In this study, two assays were developed to detect RVs using an RT-LAMP method with primers that were designed based on recently circulating RVs in Japan. Among nucleic acid amplification

TABLE 5 The performance of the RT-LAMP assays compared with real-time RT-PCR assay on RV-C-positive specimens

Real-time RT-PCR	RT-LAMP		Specificity; 95% CI	Sensitivity; 95% CI
	Positive	Negative		
Positive	17	5	100.0%;88.4%-100.0%	77.3%;55.6%-92.2%
Negative	0	30		

Abbreviations: CI, confidence interval; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; RV, rhinovirus.

techniques, real-time fluorescent RT-LAMP can be performed simply by incubating the reaction tube at 63°C and completed within 30 minutes.[22] Other than real-time RT-PCR and RT-LAMP methods, nucleic acid sequence-based amplification (NASBA) to detect RVs, has been reported.[27-30] Similar to RT-LAMP, NASBA is an isothermal nucleic acid amplification method that can be performed without the need for expensive equipment. However, NASBA takes 2 hours to generate a result, and requires an annealing step at 65°C before an isothermal amplification step at a lower temperature.[31] It has been more than a decade since NASBA assays for RV detection were reported, and it is still not certain whether these assays can detect recently circulating RVs, especially the recently identified RV-Cs. More recently, reverse transcription strand invasion-based amplification (RT-SIBA), which can be performed isothermally to detect RVs, has been reported[32]; however, this assay takes more than 1 hour to generate results and has only been evaluated with RV-A and -B, but not with RV-C. Similar to NASBA assays, it is uncertain whether RT-SIBA can detect RV-Cs.

RV genomes are highly diverse between different species, as well as within the same species; therefore, molecular assays typically target the 5′-UTR of the viral genome, which contains relatively conserved sequences.[7,33] Our developed RT-LAMP assays also target the 5′-UTR and had no cross-reactivity with other tested enteroviruses and detected 19 out of 21 tested RV-A species and seven out of eight tested RV-C species (Table 2and 3). In contrast, the assays failed to detect two RV-A, 1 RV-B, and 1 RV-C species (Tables 2 and 3), which may have been caused by the presence of mismatches between the target RNA and the primers. Focusing on the QPrimer region, the four undetectable viruses have three nucleotide mismatches at the 3′-end of the Set 1 QPrimer and one nucleotide mismatch at the 3′-region of the Set 2 QPrimer. Meanwhile, from the assay results showing a detection rate of 90% for the tested RVs with high sensitivity and specificity (Tables 2 and 3), the assays were considered to be useful as molecular detection tools for RV infections.

Our developed RT-LAMP assays have a sensitivity of 86.3% and 77.3% for detecting RV-A and -C, respectively, in clinical specimens when compared with real-time RT-PCR (Tables 4 and 5). Unfortunately, only three RV-B-positive clinical specimens were available and the assays failed to detect all of them. The Cp values of the real-time PCR assays for these three clinical specimens were greater than 30 (data not shown), so it was speculated that the low concentrations of the viral genomes resulted in their nondetection. Considering that the inability of the assays to detect RV-Bs is a limitation of this approach, further evaluation using additional RV-B-positive clinical specimens and improvement of the assays are required. Recently, several molecular epidemiological studies have been conducted to type RVs, and it was shown that the majority of circulating RV species were RV-A and -C, and even the prevalence of each RV species differed in each report.[34-36] Considering these epidemiological studies and the sensitivity of the developed assays in the present study, it is suggested that the assays can detect 70% to 80% of recently circulating RVs.

In conclusion, both of the developed RT-LAMP assays can be performed easily and quickly, are highly specific, and can detect the majority of recently circulating RVs. The assays are considered helpful for the diagnosis of RV infections; consequently, the results will help to enhance the surveillance of respiratory illness and to study the roles of RVs associated with clinical features and disease severity.

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CONFLICT OF INTERESTS

The authors declared that there is no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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