Original Article

Detection of Respiratory Viruses from ARTI Patients by xTAG RVP Fast v2 Assay and Conventional Methods

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Abstract

Introduction: **Acute respiratory tract infections (ARTIs) are a major cause of morbidity and mortality in paediatric patients. Therefore, early detection of the viral aetiologies of ARTIs is essential for patient management and infection control. In this study, we evaluated the performance of a new multiplex polymerase chain reaction (PCR) assay (xTAG Respiratory Viral Panel [RVP] Fast v2) in the detection of respiratory viruses by comparing it with that of viral culture and direct immunofluorescence (IF) staining.**

Methods: **Nasopharyngeal swab and aspirate samples were collected prospectively from 199 patients who presented with ARTIs at the University Malaya Medical Centre (UMMC) in Kuala Lumpur, Malaysia during a 10-month period. The PCR assay was conducted in parallel with conventional culture and direct IF staining methods.**

Results: **The positive rate of the xTAG RVP Fast v2 assay (78.4%) in detecting respiratory viruses was higher than that of the viral isolation (7.5%) and direct IF (23.1%) methods. Using the xTAG RVP Fast v2 assay, human enterovirus/human rhinovirus (HEV/HRV) was the most frequently detected (46.2%). The xTAG RVP Fast v2 assay revealed mixed infection caused by two or three respiratory viruses in 40 specimens, and these were undetected by the viral isolation and direct IF methods.**

Conclusion: **The xTAG RVP Fast v2 assay was superior to conventional methods in the identification of common respiratory viruses, with higher sensitivity and shorter turnaround times for laboratory results.**

Keywords: *respiratory tract infections, viruses, Multiplex PCR, diagnostic tests, routine*

Introduction

The worldwide emergence and transmission of respiratory viruses pose significant clinical and societal challenges. Viral respiratory infections mainly affect children under 5 years (1, 2), elderly adults and immunocompromised patients (3, 4). On average, five to eight respiratory viruses are detected in paediatric patients every year (5, 6). These viruses are well-known causes of acute respiratory tract infections (ARTIs), which are a major source of morbidity and mortality in infants and young children (7). Williams et al. (8) reported that an estimated 1.9-million children died from ARTIs worldwide in 2000, with most deaths recorded in Africa and Southeast Asia.

The diagnosis of viral respiratory infections relies on four different techniques: a) virus isolation in cell cultures, b) antigen detection, c) antibody detection (serology) and d) nucleic acid-based molecular methods (9–11). Many

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conventional methods, such as viral culture and immunofluorescence (IF) detection, are time consuming and have low sensitivity. Viral culture is recognised as the 'gold standard' for the identification of viral pathogens. However, cytopathic effects in cell cultures may not be observed for up to 10 days in diagnostic laboratories. Although a combination of viral culture and IF staining is sufficient to obtain a positive result, these methods are associated with a large number of false negative results (12–14).

Various monoplex polymerase chain reaction (PCR)-based techniques have been developed to overcome the limitations of low sensitivity and slow turnaround times of current detection methods. However, monoplex PCR assays require separate amplification of each virus of interest. Thus, the development of highly sensitive and specific multiplex molecular assays is needed to provide a cost-effective method of diagnosis and improve clinical management of viral respiratory infections.

The xTAG Respiratory Viral Panel (xTAG RVP) Fast v2 represents a paradigm shift in diagnostic tests. It is based on a simpler protocol and has shorter turnaround times (approximately 4 h) than conventional assays. Furthermore, it is the only assay to use the MAGPIX system. The xTAG RVP Fast v2 assay is a qualitative multiplex molecular diagnostic test, which uses a bead-based hybridisation system for simultaneous detection of 19 viruses and subtypes: respiratory syncytial virus (RSV); influenza virus A, including subtypes H1, H3 and H1N1; influenza virus B; parainfluenza virus type 1–4 (PIV-1, -2, -3 and -4); adenovirus (AdV); human enterovirus/human rhinovirus (HEV/HRV); human bocavirus (HBoV); human metapneumovirus (HMPV); and human coronavirus (HCoV) 229E, NL63, OC43 and HKU1 subtypes. The sensitivity and specificity of an older version of the RVP Fast assay was reported to be 78.8% and 99.6%, respectively, as compared to the real-time PCR assay (15).

In addition to public health concerns associated with viral respiratory infections, long-term acute care/hospitalisation and ineffective treatment can place an increased burden on the limited resources of health care services. Rapid and accurate detection of viral aetiology is important to enable early therapeutic interventions and prevent further transmission (16–18). The aim of the present study was to compare the performance of the commercially available xTAG RVP Fast v2 assay with that of routine laboratory diagnostic tests (conventional viral culture and IF staining) in the detection of respiratory viruses in patients with ARTIs.

Methods

Sample Collection

As our institute is a teaching hospital, all the respiratory specimens were obtained as part of routine diagnostic tests in the virology laboratory. Thus, the study was exempt from ethical approval (http://www.ummc.edu.my/view/content.php?ID=VGxSWlBRPT0=). The identity of all the donors remained anonymous. All information on the source samples (name, IC, laboratory number and race) was removed, with the exception of information on sex and the clinical diagnosis.

Subjects and Respiratory Specimens

Nasopharyngeal swab samples were collected from 199 outpatients and patients admitted to the paediatric ward and intensive care unit of the University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia from November 2013 to August 2014. Sociodemographic and microbiological data on each patient were recorded.

The medical records of each patient at the time of the sample collection were examined. An ARTI was defined as a new illness occurring within the previous three weeks, associated with a runny nose, cough, fever and sore throat, in addition to nasal congestion. ARTIs included upper or lower respiratory tract infections. Patients with a longer duration of symptoms were excluded. Duplicate specimens collected from the same patient within the same week were also excluded. All other samples obtained from the patients were included in the analysis, irrespective of the time of admission. Thus, nosocomial infections could not be excluded. All respiratory samples for viral isolation, direct IF and testing using the xTAG RVP FAST v2 assay were obtained at the same time. Nasopharyngeal swab and aspirate samples were transported in 3 ml of viral transport medium in a cold chain. All the samples were processed and examined immediately upon receipt at the virology laboratory.

Direct IF and Viral Isolation

All respiratory specimens were routinely screened for respiratory viruses by direct IF staining and viral culture. Each respiratory specimen was centrifuged, and the cells

were fixed onto slides. Direct IF staining for respiratory viruses (influenza A, influenza B, PIV- 1, -2 and -3, AdV, RSV and HMPV) was then conducted using the D3 Ultra DFA Respiratory Virus Screening & ID Kit (Diagnostic Hybrids, OH, USA) according to the manufacturer's protocol. Positive identification was accepted as the presence of three of more intact cells per well, with specific fluorescence.

Viral isolation was carried out as previously described (19). All respiratory specimens were inoculated into Madin-Darby canine kidney (ATCC number CCL-34), African green monkey kidney (Vero; ATCC number CCL-81), rhesus monkey kidney (LLC-MK2; ATCC number CCL-7), human lung adenocarcinoma (A549; ATCC number CCL-185) and human epithelial type 2 (ATCC number CCL-23) cells. The infected cells were harvested, and IF staining was performed as described above.

xTAG RVP FAST v2 Assay

The xTAG RVP FAST v2 assay was performed according to the manufacturer's protocol. Briefly, viral RNA/DNA was extracted from the viral transport medium using a Qiagen MinElute Virus Spin Kit (Qiagen Inc., CA, USA) and an automated QIAcube system (Qiagen Inc.) according to the manufacturer's instructions. A total of 18 µL of xTAG bacteriophage MS2 was spiked into 182 µL of sample to confirm that the whole process from nucleic acid extraction to reverse transcription steps was functioning properly.

cDNA synthesis and PCR amplification were conducted in a single-tube format. The assay was carried out in a final volume of 20 µL containing 1.3 µL of xTAG RNase-free water, 4 µL of 5× xTAG OneStep Buffer, 1.1 µL of xTAG dNTP mix, 2 µL of xTAG RVP FAST v2 Primer Mix, 1.6 µL of xTAG OneStep Enzyme Mix and 10 µL of extracted nucleic acid sample or bacteriophage lambda DNA (run control) using a Bio-Rad DNA Engine (MJ PTC 200) (Bio-Rad, Hercules, CA, USA). The amplification conditions were as follows: 50 °C for 20 min, initial denaturation at 95 °C for 15 min, followed by 36 cycles of denaturation at 95 °C for 30 sec, annealing at 59 °C for 30 sec and extension at 72 °C for 30 sec. Finally, the PCR process was ended by a final extension at 72 °C for 2 min.

Hybridisation was performed by mixing 2 µL of amplicons with 20 µL of xTAG RVP FAST v2 Bead Mix and 75 µL of reporter solution. The mixture was incubated at 45 °C for 20 min in the Bio-Rad DNA Engine (MJ PTC 200). Virus detection was performed using the xMAP instrument (Luminex, Austin, TX, USA) (20).

Statistical Analysis

Statistical analysis was performed using Microsoft Excel. Categorical variables were summarised using frequencies and percentages.

Results

Patient demographics and major findings are summarised in Table 1. The age of the patients ranged from 4 months to 62 years, with a mean age of 3.7 years. There were only four adult patients (2.01%), including one elderly individual. The mean age of the other 195 patients was three years. The median age of the study population was two years. The male to female ratio was 1:4.

Of the 199 respiratory specimens, 15 (7.5%) were positive by viral culture for RSV, AdV, influenza A H3 subtype, influenza B and PIV-2 (Table 1). A total of 46 specimens (23.1%) were positive by direct IF (Table 1); the viruses detected included RSV, AdV, influenza A H3 subtype, PIV-1 and PIV-3. The majority of the respiratory specimens (78.4%, 156/199) were positive according to the xTAG RVP FAST v2 assay (Table 1). The assay identified RSV, HRV/HEV, AdV, influenza A H3 subtype, influenza A H1N1 subtype, influenza B, PIV-1, PIV-2, PIV-3, PIV-4, HBoV, HCoV, NL63 subtype and HMPV. None of the specimens were positive for influenza A H1 subtype or HCoV NL63, HKU1 and 229E subtypes. According to all three methods, eight specimens were positive (AdV [n = 1], influenza A [n = 1] and RSV [n = 6]), and 43 specimens were negative (Table 2). The results of viral isolation and direct IF revealed discrepancies in findings for 44 specimens, with RSV (n = 29), PIV-3 (n =2), HMBV (n = 5) and influenza A (n = 1) detected by direct IF but not isolated by the culture method. Conversely, RSV (n = 1), AdV (n = 2), influenza A (n = 1), influenza B (n = 2) and PIV-2 (n = 1) were isolated by the culture method but not by direct IF.

Based on the findings of the viral culture and xTAG RVP Fast v2 assay, the results of 57 specimens were incongruent (14 positive specimens and 43 negative specimens). According to the viral culture method, 14 specimens were positive: RSV (n = 7), AdV (n = 3), influenza A (n = 2), influenza B (n = 1) and PIV-2 (n = 1). In contrast, the positive specimens according to the xTAG RVP Fast v2

assay were RSV (n = 5), AdV (n = 2), influenza A H3 subtype (n = 1), influenza A H1N1 subtype (n = 1) and influenza B (n = 1), with four respiratory specimens found to have mixed infections (Table 3). Of the 142 inconsistent results, the xTAG RVP Fast v2 assay identified viruses from all the culture-negative specimens (141). Viral culture is expected to be able to isolate 68 single respiratory viruses, except PIV-4, HBoV, HCoV (subtypes OC43, 229E and NL63), HEV and HRV, which are unculturable (Table 3). The results of the viral isolation and xTAG RVP Fast v2 assay methods were also discordant for one specimen, with the xTAG RVP Fast v2 assay identifying HRV/HEV and viral isolation identifying the influenza B virus.

Direct IF detected 45 positive specimens: RSV (n = 35), HMPV (n = 5), AdV (n = 1) influenza A (n = 2), PIV-1 (n = 1) and PIV-3 (n = 1), as shown in Table 4. In contrast, the viruses detected using the xTAG RVP Fast v2 assay were as follows: RSV (n = 27), HMPV (n = 4), AdV (n = 1), influenza A H3 subtype (n = 1), influenza A H1N1 subtype (n = 1), PIV-1 (n = 1) and PIV-3 (n = 1), with nine respiratory specimens found to have mixed infections. In addition, the xTAG RVP Fast v2 assay detected respiratory viruses in all 111 (55.8%, 111/199) direct IF-negative specimens. Among these, 18 viruses (RSV [n = 5], HMPV [n = 5], AdV [n = 1], PIV-1 [n = 2], PIV-3 [n = 1], influenza A H3 subtype [n = 1], influenza A H1N1 subtype [n = 1], and influenza B [n = 1]) should have been detected by direct IF (Table 4).

Table 1. The basic characteristics of patients from November 2013 to August 2014

Patient characteristics	Frequency (%), n=199
Sex ratio (male : female)	1.4 (117: 82)
Age [total number (percentage)]	
0–1	24 (12.1%)
<1–5	140 (70.3%)
>5–21	31 (15.6%)
>21–65	3 (1.5%)
>65	1 (0.5%)
Results [total number (percentage)]	
Number of viral culture positive specimens	15 (7.5%)
Number of direct IF positive specimens	46 (23.1%)
Number of xTAG RVP Fast v2 assay positive specimens	156 (78.4%)

Table 2. Concordance between viral isolation, direct IF and xTAG RVP Fast v2 assay for the detection of respiratory viruses

Target virus	viral isolation	Direct IF	xTAG RVP Fast v2	Frequency (%)
AdV	+	+	+	1 (0.5)
Influenza A	+	+	+	1 (0.5)
RSV	+	+	+	6 (3.0)
Total				8 (4.0)
No virus detected	-	-	-	43 (21.6)

+: virus detected
-: no virus detected

Table 3. Distribution of specimens isolated by viral isolation or detection by xTAG RVP Fast v2 assay

	No. of specimens with respiratory viruses isolated by viral culture (*n*)a	No. of specimens with single virus detected by xTAG RVP Fast v2 assay (*n*)a	No. of specimens with multiple viruses detected by xTAG RVP Fast v2 assay (*n*)a	Total no. of specimens with respiratory viruses detected by xTAG RVP Fast v2 assay (%)
Consistent (57, 28.6%)	RSV (7)	RSV (5)	RSV/HBoV (1) RSV/AdV (1)	7 (3.5)
	AdV (3)	AdV (2)	AdV/HRV/HEV (1)	3 (1.5)
	Influenza A (2)	Influenza A H3 subtype (1)	-	2 (1.0)
		Influenza A H1N1 subtype (1)		
	Influenza B (1)	Influenza B (1)	-	1 (0.5)
	PIV-2 (1)	-	PIV-2/HBoV (1)	1 (0.5)
	Negative (43)	Negative (43)		43 (21.6)
Inconsistent (142, 71.4%)	Influenza B (1)	HRV/HEV (1)	-	1 (0.5)
	Negative (141)	HRV/HEV (58) RSV (27) HMPV (9) PIV-1 (3) HBoV (2) PIV-3 (2) PIV-4 (1) Influenza A H3 subtype (1) Influenza A H1N1 subtype (1) Influenza B (1)	HRV/HEV/HBoV (12) HRV/HEV/AdV (7) HRV/HEV/RSV (4) HRV/HEV/HMPV (3) HRV/HEV/PIV-1 (3) HRV/HEV/PIV-2 (1) HRV/HEV/PIV-3 (1) HBoV/RSV (1) RSV/PIV-4 (1) RSV/AdV (1) RSV/HCoV NL63 subtype (1) HRV/HEV/RSV/AdV (1)	141 (70.9)

aAdV, adenovirus; HRV, human rhinovirus; HBoV, human bocavirus; HEV, human enterovirus; RSV, respiratory syncytial virus; PIV, parainfluenza virus; HMPV, human metapneumovirus.

Table 4. Distribution of specimens detected by direct IF or xTAG RVP Fast v2 assay

	No. of specimens with respiratory viruses detected by direct IF staining of clinical specimens (*n*)a	No. of specimens with single virus detected by xTAG RVP Fast v2 assay (*n*)a	No. of specimens with multiple viruses detected by xTAG RVP Fast v2 assay (*n*)a	Total no. of specimens with respiratory virus detected by xTAG RVP Fast v2 assay (%)
Consistent (88, 44.2%)	RSV (35)	RSV (27)	RSV/HRV/HEV (3) RSV/HBoV (2) RSV/AdV (2) RSV/PIV 4 (1)	35 (17.6)
	AdV (1)	AdV (1)	-	1 (0.5)
	Influenza A (2)	Influenza A H3 subtype (1) Influenza A H1N1 subtype (1)	-	2 (1.0)
	HMPV (5)	HMPV (4)	HMPV/HRV/HEV (1)	5 (2.5)
	PIV-1 (1)	PIV-1 (1)	-	1 (0.5)
	PIV-3 (1)	PIV-3 (1)	-	1 (0.5)
	Negative (43)	Negative (43)		43 (21.6)
Inconsistent (111, 55.8%)	Negative (111)	HRV/HEV (59) RSV (5) HMPV (5) PIV-1 (2) HBoV (2) Influenza B (2) PIV-3 (1) PIV-4 (1) Influenza A H3 subtype (1) Influenza A H1N1 subtype (1) AdV (1)	HRV/HEV/HBoV (12) HRV/HEV/AdV (8) HRV/HEV/RSV (1) HRV/HEV/HMPV (2) HRV/HEV/PIV-1 (3) HRV/HEV/PIV-2 (1) HRV/HEV/PIV-3 (1) PIV-2/HBoV (1) RSV/HCoV NL63 subtype (1) HRV/HEV/RSV/AdV (1)	111 (55.8)

aAdV, adenovirus; HRV, human rhinovirus; HBoV, human bocavirus; HEV, human enterovirus; RSV, respiratory syncytial virus; PIV, parainfluenza virus; HMPV, human metapneumovirus.

The overall distribution of the respiratory viruses in the 199 patients identified by the viral isolation, direct IF and xTAG RVP Fast v2 assay methods is shown in Table 5. In total, 197 viruses were detected in the 156 xTAG RVP Fast v2 assay-positive specimens. In contrast, only a single virus was detected in each of the 15 and 46 viral culture- and direct IF-positive specimens. RSV was commonly detected using the routine laboratory diagnostic tests (viral isolation and direct IF). In the 199 specimens, the xTAG RVP Fast v2 assay, direct IF and viral isolation methods detected 43 (21.6%), 35 (17.6%) and 7 (3.5%) RSV-positive specimens, respectively. Using the xTAG RVP Fast v2 assay, HEV/HRV was the most frequently detected agent (present in 46.2% of all samples from the 199 patients) (Table 5). Diagnostic tests for HEV and HRV are not routinely performed in our laboratory unless requested by a clinician. In this study, none of the respiratory specimens had undergone testing for HEV and HRV. The xTAG RVP Fast v2 assay also identified viruses that would not have been detected by viral isolation and direct IF: HBoV (8.5%, 17/199), PIV-4 (1.0%, 2/199) and HCoV NL63 subtype (0.5%, 1/199), as shown in Table 5. Furthermore, the xTAG RVP Fast v2 assay was able to detect subtypes of influenza A (two influenza A H3 subtypes and two influenza A H1N1 subtypes) (Table 5).

The xTAG RVP Fast v2 assay identified single virus infection in 116 (58.3%) of the 199 specimens, and it detected two or more respiratory viruses in 40 (20.1%) of the 199

specimens. Of these 40 specimens, it detected three respiratory viruses (HRV/HEV, RSV and AdV) in one respiratory sample and two respiratory viruses in the other 39 samples. Most cases of mixed infection were caused by HEV/HRV (Table 5), which was found in association with RSV (2.0%, 4/199), AdV (4.0%, 8/199), PIV-1 (1.5%, 3/199), PIV-2 (1.0%, 1/199), PIV-3 (1.0%, 1/199), HBoV (12%, 6/199) and HMPV (1.5%, 3/199). There were no cases of mixed infection involving influenza A and influenza B viruses. The majority of multiple respiratory viral infections (92.5%, 37/40) were detected in children aged < 5 years.

Table 5. Distribution of respiratory viruses identified by xTAG RVP Fast v2 assay, viral isolation and direct IF

Target virus	Virus subtype	Total respiratory virus detected by xTAG RVP Fast v2 assay (%)	Mono-infection (%)	Co-infection (%)	Total positive specimen number detected by viral isolation (%)	Total positive specimen number detected by direct IF (%)a
RSV		43 (21.8)	32 (16.2)	11 (5.6)	7 (3.5)	35 (17.6)
HRV/HEV		92 (46.2)	59 (29.9)	33 (16.8)	-	-
AdV		13 (6.6)	2 (1.0)	11 (5.6)	3 (1.5)	1 (0.5)
Influenza A	H3	2 (1.0)	2 (1.0)	-	2 (1.0)a	2 (1.0)a
	H1N1 (2009)	2 (1.0)	2 (1.0)	-		
Influenza B		2 (1.0)	2 (1.0)	-	2 (1.0)	-
PIV	1	6 (3.0)	3 (1.5)	3 (1.5)	-	1 (0.5)
	2	2 (1.0)	-	2 (1.0)	1 (0.5)	-
	3	3 (1.5)	2 (1.0)	1 (0.5)	-	2 (1.0)
	4	2 (1.0)	1 (0.5)	1 (0.5)	-	-
HBoV		17 (8.6)	2 (1.0)	15 (7.6)	-	-
HCoV	NL63	1 (0.5)	-	1 (0.5)	-	-
HMPV		12 (6.1)	9 (4.6)	3 (1.5)	-	5 (2.5)
Total		197 (100)	116 (58.9)	81 (41.1)	15 (7.5)	46 (23.1)

[a] Viral culture and direct IF staining of clinical specimens does not differentiate influenza A subtype. The number of 2 means the counting of influenza A irrespective of any Influenza A subtype.

Discussion

The majority of ARTIs in developed countries have been reported to be caused by RSV, influenza viruses, PIV and AdV (1, 21, 22). Patients with ARTIs are treated symptomatically as outpatients. Respiratory tract viruses cannot be identified based on symptoms alone due to the similarity in clinical presentation of ARTIs. Therefore, treatment is usually initiated without aetiological identification or treated ineffectively with antibacterial agents based on suspected bacterial causes (23). In addition, the clinical features of RSV infection are difficult to distinguish from those of infections caused by the influenza virus or other respiratory viruses (4). According to Weinberg (24), patients found to have PIV-4 infection were less symptomatic than those with other types of respiratory infections. HBoV and HRV were also reported to be associated with an asymptomatic presentation (25, 26).

The existence of a number of new respiratory viruses, such as HMPV (27), mimivirus (28), HCoV-NL63 (29), HCoV-HKU1 (30) and HBoV (31), all of which were identified between 2001 and 2005, poses a formidable challenge. The lack of information about these viruses makes rapid diagnosis of ARTIs even more important. The emergence of the swine flu pandemic (32) and influenza B outbreak (33) in the U.K. emphasises the importance of rapid and accurate detection of respiratory viruses, which can then be treated with antiviral drug therapy.

The xTAG RVP Fast v2 assay is a straightforward assay as compared to conventional methods. Importantly, the findings of the present study demonstrated that the positive rate of detection of major respiratory viruses (RSV, AdV, influenza viruses and PIV) using the xTAG RVP Fast v2 assay was higher than that of the conventional viral isolation and direct IF methods. As previously reported, the diagnosis of RSV infection is difficult due to insensitive detection methods (viral culture and antigen detection) (4). The viability and low titre of respiratory viruses in specimens might explain the failure of conventional methods to detect respiratory viruses in xTAG RVP Fast v2 assay-positive specimens.

HBoV, HCoV and HRV are associated with upper respiratory tract infections (34–36). The ability of the xTAG RVP Fast v2 assay to detect these viral pathogens, as well as PIV-4 and the influenza A H1N1 subtype, which has caused severe infections and outbreaks worldwide (37, 38), is an advantage of multiplex molecular assays over conventional diagnostic methods. In addition, the xTAG RVP Fast v2 assay can detect respiratory viruses, such as HMBV and HRV, both which are difficult to culture in cell lines (27, 39). A cost analysis previously performed on a paediatric population demonstrated that the RVP assay was a much less costly strategy than viral culture and IF staining methods for the diagnosis of viral respiratory tract infections (40). Therefore, this advanced multiplex test can be implemented routinely in hospital laboratories. However, an intrinsic limitation of the RVP Fast assay is cross-reactivity of primers with HEV because of the genetic similarity between these two viruses.

In this study, multiple respiratory viruses were identified in 40 respiratory specimens. Most of the co-infections involved HEV/HRV (11.1%, 22/199). Noh et al. (41) reported that HRV was the most frequently identified concurrent respiratory virus in influenza patients during the 2011–2012 season. However, in the present study, influenza viruses occurred mainly as single infectious agents of ARTIs. In contrast, HEV/HRV most commonly occurred in conjunction with HBoV in children. The clinical significance of viral co-infections remains uncertain. Noh et al. (41) showed that the severity of clinical illness in patients with multiple viral agents was not markedly different from that of patients with a single viral agent. However, in some cases, respiratory viruses in co-infections have been reported to cause more severe illness than the viral pathogens responsible for the primary infection. In the present study, it was not possible to differentiate between the primary infectious virus and the co-infecting virus. A further study to correlate the clinical significance of the presence of multiple viral pathogens could be performed in the future.

In conclusion, our results highlighted the superiority of the xTAG RVP Fast v2 assay in the diagnosis of viral respiratory infections and co-infections. In comparison to conventional viral isolation and direct IF methods, this assay is rapid and highly sensitive and can be used in routine respiratory disease diagnostic services. The clinical use of the xTAG RVP Fast v2 assay will aid the diagnosis of infection, thereby improving clinical management via earlier treatment. However, prior to the routine application of the xTAG RVP Fast v2 assay in diagnostic virology laboratories, the analytical sensitivity of the assay must be considered in relation to hands-on-time, time-to-results and clinical relevance of the detected respiratory viruses.

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Authors' Contributions

Conception and design: CSK, SMY, KPN
Analysis and interpretation of the data: CSK, SMY, KPN
Drafting of the article: CSK, SMY, KPN
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