Original Article

Utility of the Xpert Xpress CoV-2/Flu/RSV Plus Kit: A Glance at RSV Infection in Adults and Coinfection Rate

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Abstract

Background: Viral infection is responsible for the majority of respiratory tract infections (RTI). This retrospective study evaluates the advantages of using the multiplex Xpert Xpress CoV-2/Flu/RSV plus kit in laboratory diagnosis of RTI caused by the most related viruses.

Materials and Methods: Data were obtained from medical records between November 2021 and May 2023 for any sample tested using either the Xpert Xpress Flu/RSV kit, Xpert Xpress Flu kit, Xpert Xpress SARS-CoV-2 kit, and Xpert Xpress CoV-2/Flu/RSV plus kit.

Results: Influenza virus A was detected in 8.5% (55/649) of the samples using the Flu PCR kit and in 4.9% (123/2538) using CoV-2/Flu/RSV plus kit, while influenza virus B detection rates were 3.7% (24/649) using the Flu PCR kit and 1.7% (43/2538) using the CoV-2/Flu/RSV plus kit. However, the detection rates using the two kits were comparable when evaluated for the same time period of the year. SARS-CoV-2 infections were detected in 16.9% (1545/9153) and 10.5% (266/2538) of the cases using the SARS-CoV-2 kit and CoV-2/Flu/RSV plus kits, respectively. Respiratory syncytial virus (RSV) was identified in 17.7% (66/372) of children using Flu/RSV kit; this number dropped significantly when age-targeted testing of RSV was performed due to involvements of adults. With the CoV-2/Flu/RSV plus kit, about 34% (35/103) of RSV infections detected were in patients aged >20 years; these cases would have previously been overlooked because adults are not routinely tested for RSV using the Flu/RSV kit. All coinfection cases (n = 16) were only detected with the CoV-2/Flu/RSV plus kit.

Conclusion: The use of Xpert Xpress CoV-2/Flu/RSV plus kit not only results in shorter turnaround times through accurate detection of all four viruses, but also provides information on RSV infection in adults and coinfection rates.

Keywords: Coinfection, human influenza, multiplex PCR, rapid diagnostic tests, respiratory tract infection, RSV, SARS-CoV-2

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INTRODUCTION

Respiratory infections, specifically lower respiratory tract infections (LRTIs), are one of the principal causes of morbidity and mortality globally. It was estimated that 1.4 million deaths in children aged <5 years are due to LRTIs, which accounts for about 18% of mortality in this age group.[1] According to the Global Burden of Disease Study 2019,[2] LRTIs ranked second for disability-adjusted life

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years (DALY) among children aged <10 years. In addition, they are among the top leading causes of DALYs among the elderly aged >75 years.[2] In 2019, the Middle East and North Africa region recorded 34.1 million cases of LRTIs.[3]

Pathogens such as viruses, bacteria, and fungi can cause respiratory infections, with viruses being the most commonly encountered. The World Health Organization has globally monitored influenza virus and respiratory syncytial virus (RSV) outbreaks since their introduction. Influenza viruses pose a great health threat to young children and the elderly worldwide, leading to 4–8.8 deaths per 100,000 individuals globally.[4] RSV caused approximately 33 million respiratory infections worldwide in 2019 and was responsible for hospitalization of >5000 children aged <5 years.[5] Since the outbreak of the SARS-CoV-2 in 2019, >600 million people have been infected, resulting in >6 million deaths worldwide.[6]

Laboratory testing is the mainstay in confirming a diagnosis of respiratory infections. For this, there are various sensitive and specific tests, but polymerase chain reaction (PCR) methods are particularly sensitive and specific. Commercial kits are available that detect individual pathogens; however, targeting each pathogen separately requires costly assays that make choosing between potential targets difficult, given that some symptoms overlap between pathogens.

The Xpert Xpress CoV-2/Flu/RSV plus kit from Cepheid (California, USA) is a multiplex PCR-based kit designed to simultaneously detect clinically important respiratory viruses in one reaction: influenza virus A and B, SARS-CoV-2, and RSV. Utilizing this assay has many expected advantages over individual tests, such as simultaneous detection of multiple viruses within one assay and coinfection detection within patients as well as increased efficiency in detecting RSV infections among older adults at lower costs than conventional tests. This study investigates the usefulness and advantages of using multiplex assays in diagnostic laboratories versus other individual assays.

MATERIALS AND METHODS

Study design, setting, and patients

This retrospective study included patients who were tested for respiratory infections at King Fahd Hospital of the University, Al Khobar, Saudi Arabia, between November 01, 2021, and May 16, 2023. Data were compiled using the electronic patient medical records and included demographic information such as age, gender, and nationality.

The study was conducted after obtaining approval from the Institutional Review Board of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia.

Diagnostic kits

In the initial 12 months of the study (i.e., between November 1, 2021, and November 14, 2022), the following three kits were used at the hospital's microbiology laboratory to detect influenza virus A and B (Flu A and B), RSV, and SARS-CoV-2: Xpert Xpress Flu/RSV kit (which detects Flu A and B and RSV); Xpert Xpress Flu kit (which identifies Flu A and B and the 2009 H1N1 Influenza virus [2009 H1N1]); and Xpert Xpress SARS-CoV-2 kit (which detects SARS-CoV-2) (Cepheid, California, USA). Thereafter (i.e., November 15, 2022, until May 16, 2023), only the Xpert Xpress CoV-2/Flu/RSV plus kit (Cepheid, California, USA) was used to detect these viruses [Figure 1]. All kits were used on the GeneXpert System (Cepheid, California, USA).

To allow comparison with the CoV-2/Flu/RSV plus kit, data from the 6 months of the previous corresponding period for each of the other kits are also reported separately.

Samples

Samples in these assays include nasal or oropharyngeal swabs in a virus transport medium for testing. The test for patients was entirely decided by the treating clinician during requesting the test order in the hospital system. We assume that the Flu/RSV kit is employed more in the case of children, as indicated by the tested age groups (see Results) because it can detect RSV, while the Flu kit was more commonly used in older ages because it tests for all influenza viruses, including H1N1. All patients were tested once by a single kit. In case of retesting during the same hospital visit, the case was considered as one provided having the same result. In the case of discrepancy, the first testing event was included in the study, as the second event was considered a follow-up. In the case of testing in two different hospital visits, both testing events were included in the study.

Statistical analysis

All data were tabulated using the Microsoft Excel software spreadsheets. The frequency of virus detection across assays and its correlation to demographic data were calculated and compared. Finally, coinfection rates were estimated from multiplex kits and statistical significance was determined using the OpenEpi website employing two by two tables and the Chi-square for linear trend test to measure statistical correlation of infection between

males and females and with age groups. *P* value <0.05 was considered significant.

RESULTS

The number of samples tested using each kit was as follows: Xpert Xpress CoV-2/Flu/RSV plus kit, 2538; Xpert Xpress Flu/RSV kit, 372; Xpert Xpress Flu kit, 649; and Xpert Xpress SARS-CoV-2 kit, 9153.

Influenza virus A and B

Flu A was detected in 7.8% (29/372) of the cases using the Flu/RSV kit and in 8.5% (55/649) of the cases using the Flu kit. In addition, it was detected in 4.9% (123/2538) of the cases using the CoV-2/Flu/RSV plus kit, and in the corresponding period, in 4.3% (6/139) of the cases using the Flu kit [Table 1]. There was no significant difference in the frequency of Flu A detection among males and females with all kits (*P* = 0.224, 0.452, and 0.182, respectively) [Tables 2–4].

Similarly, Flu B was detected in 1.3% (5/372) and 3.7% (24/649) of the cases using the Flu/RSV kit and the Flu kit, respectively. Further, it was detected in 1.7% (43/2538) of the cases using the CoV-2/Flu/RSV plus kit, and in the corresponding period, in 2.2% (3/139) of the cases using the Flu kit [Table 1]. No significant difference was found in the Flu B infection rates between males and females using any of the kits (*P* = 0.548, 0.582, and 0.972, respectively) [Tables 2–4].

Respiratory syncytial virus

RSV was detected in 17.7% (66/372) of the cases using the Flu/RSV kit. In addition, it was detected in 4.1% (103/2538) of the cases using the CoV-2/Flu/RSV plus kit, and in the corresponding period, in 21.2% (48/222) of the cases using the Flu/RSV kit [Table 1]. There was no significant difference in the detection rates between males and females with both kits (*P* = 0.972 and 0.073,

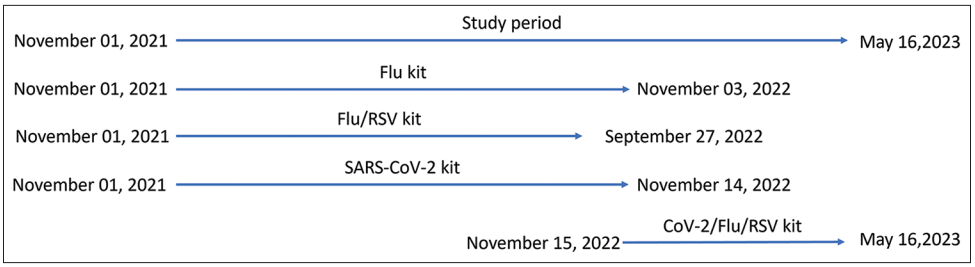

Figure 1: The use of various kits across the study period

Table 1: The frequency and percentage of detection of each virus by each kit during the indicated period of the study

Kit name	November 2021–November 2022			November 2021–May 2022			November 2022–May 2023
	Target virus						
	Flu/RSV	Flu PCR	SARS-CoV-2	Flu/RSV	Flu PCR	SARS-CoV-2	CoV-2/Flu/RSV plus
Number of tested cases	372	649	9153	222	139	5669	2538
Flu A	29 (7.8)	55 (8.47)	ND	4 (1.8)	6 (4.32)	ND	123 (4.85)
Flu B	5 (1.34)	24 (3.7)	ND	3 (1.14)	3 (2.16)	ND	43 (1.69)
RSV	66 (17.7)	ND	ND	48 (21.2)	ND	ND	103 (4.06)
H1N1	ND	0 (0)	ND	ND	0 (0)	ND	ND
SARS-CoV-2	ND	ND	1545 (16.88)	ND	ND	938 (17.3)	266 (10.48)

ND – Not detectable by the kit; PCR – Polymerase chain reaction; RSV – Respiratory syncytial virus

Table 2: Distribution of positive cases detected by the Flu/RSV kit based on gender and age

Parameters	Number of tested cases	Flu A		Flu B		RSV	
		Positive, *n* (%)	*P*	Positive, *n* (%)	*P*	Positive, *n* (%)	*P*
Male	203	19 (9.36)	0.224	2 (0.99)	0.548	37 (18.23)	0.972
Female	169	10 (5.92)		3 (1.78)		29 (17.16)	
Total	372	29 (7.80)		5 (1.34)		66 (17.74)	
Age groups (years)							
0–10	351	27 (7.69)	0.526	5 (1.42)	0.460	66 (18.80)	0.074
11–20	13	2 (15.38)		0		0	
21–30	1	0		0		0	
31–40	1	0		0		0	
41–50	2	0		0		0	
51–60	2	0		0		0	
>60	2	0		0		0	
Total	372	29 (7.80)		5 (1.34)		66 (17.74)	

RSV – Respiratory syncytial virus

respectively) [Tables 2 and 4]. No cases of 2009 H1N1 were reported during the study [Table 3].

SARS-CoV-2

SARS-CoV-2 was detected in 16.9% (1545/9153) of the cases using the singleplex SARS-CoV-2 kit; the rate was similar (938/5669; 17.3%) between November 15, 2021, and May 16, 2022 [Table 1]. However, SARS-CoV-2 detection rate decreased to 10.9% (266/2538) when using the CoV-2/Flu/RSV plus kit from November 2022 to May 2023 [Table 1]. In addition, the infection rates were found to be significantly higher in males than females using both kits (SARS-CoV-2 kit: $P = 0.001$, 95% confidence interval: 1.67–1.00; CoV-2/Flu/RSV plus kit: $P = 0.048$, 95% confidence interval: 1.53–1.91) [Tables 4 and 5].

Age-based infection rates

The average age for individuals tested was 4.3 years with the Flu/RSV kit, 25.3 years with the Flu kit, 33.9 years with the SARS-CoV-2 kit, and 29.7 years with the CoV-2/Flu/RSV plus kit. As shown by Tables 2–4, no statistically significant difference was noted among different age groups for Influenza A or B infection using any of the kits [Tables 2–4]. Expectedly, RSV infection was more prevalent among children than in other age groups [Tables 2 and 4]. SARS-CoV-2 infections were more likely to be detected among higher age groups [Tables 4 and 5].

Coinfection

Coinfection with two or more viruses was reported in 16 of 535 positive cases with the CoV-2/Flu/RSV plus kit. Most coinfection cases were among those aged 41–50 years; all infections were in patients aged ≤50 years [Table 6]. No coinfection cases were noted with other kits; most incidents occurred during winter (November to January).

DISCUSSION

This study compares the detection frequency of the most clinically relevant respiratory viruses, namely, influenza virus A, influenza virus B, RSV, and SRAS-CoV-2, with four PCR-based assays over an 18-month period. Of these, the CoV-2/Flu/RSV plus kit is the only multiplex kit that detects all four viruses in one reaction and was used during the last 6 months of the study.

The detection rate of influenza viruses A and B was reduced between November 2022 and May 2023 using the CoV-2/Flu/RSV plus kit compared with the detection rate in the previous period. It is most probably due to missing some winter months (September and October) of using

Table 3: Distribution of positive cases detected by the Flu kit based on gender and age

Parameters	Number of tested cases	Flu A		Flu B		2009 H1N1 Flu
		Positive, *n* (%)	*P*	Positive, *n* (%)	*P*	Positive, *n* (%)
Male	315	24 (7.62)	0.452	13 (4.13)	0.582	0
Female	334	31 (9.28)		11 (3.29)		0
Total	649	55 (8.47)		24 (3.70)		0
Age groups (years)						
0–10	301	25 (8.31)	0.699	14 (4.65)	0.081	0
11–20	51	4 (7.84)		1 (1.96)		0
21–30	58	3 (5.17)		4 (6.90)		0
31–40	77	7 (9.09)		2 (2.60)		0
41–50	41	4 (9.76)		2 (4.88)		0
51–60	36	3 (8.33)		1 (2.78)		0
>60	83	8 (9.64)		0		0
Total	647	54 (8.35)		24 (3.71)		0

Table 4: Distribution of positive cases detected by the CoV-2/Flu/RSV plus kit based on gender and age

Parameters	Number of tested cases	Flu A		Flu B		RSV		SARS-CoV-2	
		Positive, *n* (%)	*P*	Positive, *n* (%)	*P*	Positive, *n* (%)	*P*	Positive, *n* (%)	*P*
Male	1305	56 (4.29)	0.182	22 (1.69)	0.972	44 (3.37)	0.073	152 (11.65)	0.048
Female	1233	67 (5.43)		21 (1.70)		59 (4.79)		114 (9.25)	
Total	2538	123 (4.85)		43 (1.69)		103 (4.06)		266 (10.48)	
Age groups (years)									
0–10	667	36 (5.40)	0.441	14 (2.10)	0.092	68 (10.19)	<0.001	22 (3.30)	<0.001
11–20	145	4 (2.76)		2 (1.38)		0		7 (4.83)	
21–30	373	16 (4.29)		6 (1.61)		8 (2.14)		51 (13.67)	
31–40	482	27 (5.60)		12 (2.49)		7 (1.45)		80 (16.60)	
41–50	265	16 (6.04)		4 (1.51)		10 (3.77)		45 (16.98)	
51–60	196	11 (5.61)		3 (1.53)		3 (1.53)		16 (8.16)	
>60	409	13 (3.18)		2 (0.49)		7 (1.71)		45 (11.00)	
Total	2537	123 (4.85)		43 (1.69)		103 (4.06)		266 (10.48)	

RSV – Respiratory syncytial virus

the kit in this study, where these viruses are commonly highly prevalent. This is further evident in the fact that the infection rates of Flu A and B rates were comparable when corresponding periods were considered for both the CoV-2/Flu/RSV plus kit and the Flu kit or Flu/RSV kit. This was in line with the existing literature, where CoV-2/Flu/RSV plus kit has been found to have high agreement with other singleplex or multiplex kits.[7]

No cases of 2009 H1N1 influenza virus were reported between November 2021 and November 2022 in the study population, thereby justifying the current lack of requirement for including this specific virus in multiplex assays, such as in the CoV-2/Flu/RSV plus kit.

The infection rates of SARS-CoV-2 were higher using the SRAS-CoV-2 kit than the CoV-2/Flu/RSV plus kit over the period of comparison, which may be explained by its higher prevalence between November 2021-2022 in population samples, which gradually diminished between November 2022–2023 due to vaccination and virus establishment in the population in addition to missing some winter months, as mentioned above.

Except for SARS-CoV-2 infection rates, no significant difference was noted between males and females for any of the other viruses; however, with both kits, males were found more likely to get infected with SARS-CoV-2 than females using either kit, which is in line with previous findings.[8-11] Similarly, SARS-CoV-2 infection was more prevalent in patients >10 years, which has also been previously reported from the same region and others.[8,12]

The Flu/RSV kit results showed higher RSV infection rates than CoV-2/Flu/RSV plus kits within the corresponding period. Due to its limited usage across other age groups, the Flu/RSV kit was chosen, as it appears from the tested age groups to test RSV infection among young children, who are known to have higher RSV prevalence. This could explain the lack of statistical significance between RSV infection and age groups using this kit. The other age groups did not contain enough tested patients to account for statistical power. On the contrary, the CoV-2/Flu/RSV plus kit was used on patients of all age groups, leading to an indirect dilution of RSV infections by immune-competent adults who are not mainly a risk group of RSV infection and therefore not routinely tested for RSV. Utilizing Flu/RSV kits only for younger patients would increase the chances of missing adults infected with RSV because they are not usually tested by this kit. In contrast, CoV-2/Flu/RSV plus kits provided an accurate view of RSV infections in adults even when requested unintentionally by treating physicians. Interestingly, approximately 34% of the RSV infections in the current study occurred in patients aged >20 years. Therefore, adult infections with RSV are more prevalent than expected. Few adult studies exist regarding RSV infections, as this infection primarily poses risks to young children and the elderly.[13]

Adults could also experience RSV infection sequelae, particularly those with chronic conditions like chronic end-stage renal disease, obstructive pulmonary disease, congestive heart failure, and diabetes mellitus.[13] RSV-infected adults, whether asymptomatic or with mild symptoms, may pose a significant threat of transmission to infants, young children, and elders. Hence, having access to a multiplex kit designed specifically to detect all these viruses including RSV in populations where RSV testing is not intended, can be particularly advantageous.

Table 5: Distribution of positive cases detected by the SARS-CoV-2 kit based on gender and age

Parameters	Number of tested cases	SARS-CoV-2	
		Positive, *n* (%)	*P*
Male	4205	881 (20.95)	<0.001
Female	4948	664 (13.42)	
Total	9153	1545 (16.88)	
Age groups (years)			
0–10	2111	219 (10.37)	<0.001
11–20	584	96 (16.44)	
21–30	1402	241 (17.19)	
31–40	1976	433 (21.91)	
41–50	991	201 (20.28)	
51–60	709	112 (15.80)	
>60	1376	242 (17.59)	
Total	9149	1544 (16.88)	

Table 6: The percentages of coinfections from the number of positive cases detected using the CoV-2/Flu/RSV plus kit

Parameters	Flu A/ SARS-CoV-2, *n* (%)	Flu B/ SARS-CoV-2, *n* (%)	RSV/ SARS-CoV-2, *n* (%)	Flu A/ RSV, *n* (%)	Flu B/ RSV, *n* (%)	Flu A/flu B, *n* (%)	Flu A/RSV/ SARS-CoV-2, *n* (%)	Total of coinfections, *n* (%)
Total	3 (0.6)	3 (0.6)	2 (0.37)	5 (0.93)	1 (0.2)	1 (0.2)	1 (0.2)	16 (4.6)
Age groups (years)								
0–10			2 (0.37)	1 (0.2)	1 (0.2)	1 (0.2)		5 (0.93)
21–30		2 (0.37)		1 (0.2)				3 (0.6)
31–40	1 (0.2)						1 (0.2)	2 (0.37)
41–50	2 (0.37)	1 (0.2)		3 (0.6)				6 (1.1)

RSV – Respiratory syncytial virus

Furthermore, using the multiplex CoV-2/Flu/RSV plus kit gave an overview of the coinfection rate with more than one virus, which was not observed with other multiplex kits for several reasons. First, no kit combined SARS-CoV-2 with other viruses. Since SARS-CoV-2 was more prevalent than other viruses, it was detected as a coinfecting virus with each of the three tested viruses. Second, influenza virus and RSV coinfection was not detected with the Flu/RSV kit because of the strategy of targeting younger children for RSV testing. The CoV-2/Flu/RSV plus kit showed that this is more likely to happen in older adults, providing an additional advantage to using this kit in clinical practice. All the coinfection incidences were observed in winter, when viruses are increasingly prevalent.

While it is tempting to assume that the price per sample and the turnaround time to test for all viruses using one kit (CoV-2/Flu/RSV plus kit) will be less than using two or more kits for the same purpose, we assume that the CoV-2/Flu/RSV plus kit would be more cost and time effective.

CONCLUSION

The CoV-2/Flu/RSV plus kit gives information about RSV infection in adults who are usually not tested for RSV infection. It also provides information about coinfections by these viruses. Therefore, the CoV-2/Flu/RSV plus kit is useful for the diagnosis of respiratory tract infections in healthcare facilities.

Ethical considerations

The study was approved by the Institutional Review Board (Ref. No: IRB-2023-01-207; date: May 11, 2023) of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. Requirement for patient consent was waived owing to the study design. The study adhered to the principles of the Declaration of Helsinki, 2013.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

K.R.A was solely involved in the Conceptualization, Methodology, Data analysis, and Writing of the manuscript. The author has read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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