Clinical Evaluation of Xpert Xpress SARS-CoV-2/Flu/RSV Combination Test in China

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Abstract: During influenza season, the influenza virus and respiratory syncytial virus (RSV) can cause severe respiratory infections. The clinical manifestation of symptoms caused by COVID-19, influenza virus and RSV are very similar.We conducted the performance characteristics of the Xpert Xpress SARS-CoV-2/Flu/RSV combined assay in China and came up with the detection results of the two detection methods are almost the same for all targets, and the consistency rate is close to 100%.This indicates that the assay is of great clinical value for distinguishing infections caused by these four viruses.

***Keyword*:** gGene; Xpert; SARS-CoV-2; influenza; RSV; detection

1. Introduction

Emerging infectious diseases are regarded as a serious threat to the global economy and human health [1,2]. In late 2019, a novel coronavirus was first reported in China and quickly spread around the world [3]. The World Health Organization (WHO) officially named the novel coronavirus as SARS-CoV-2, and the pneumonia caused by the novel coronavirus was named coronavirus disease-19 (COVID-19)[4]. Due to the rapid transmission of the novel coronavirus, the number of people infected with SARS-CoV2 has exceeded 200 million [5]. During influenza season, the influenza virus and respiratory syncytial virus (RSV) can cause severe respiratory infections. The clinical manifestation of symptoms caused by COVID-19, influenza virus and RSV are very similar, however there are fundamental differences in treatment and management of each disease[6]. Distinct from common cold viruses, infection with SARS-CoV2, influenza or RSV is often accompanied by fever and other systemic symptoms, especially for the elderly, and the consequences are more serious[7]. Therefore, how to quickly distinguish between common cold, SARS-CoV2, RSV and influenza infections is critical for treatment and patient outcome.

The Xpert Xpress SARS-CoV-2/Flu/RSV combination assay, developed by Cepheid and approved for emergency use by the U.S. Food and Drug Administration in December 2020, can rapidly confirm SARS-CoV-2, influenza A and B or RSV in less than 35 minutes. Unfortunately, the product has not yet obtained approval for use in China.We have been fortunate to win the sponsorship of Saipan Company, in the molecular biology laboratory of Weifang Second People's Hospital, to test the Xpert Xpress assay and Zhongshan Da 'an Co., Ltd. SARS-CoV-2, FluA, FluB, RSV kit (RT-PCR) assay for direct comparison and to evaluate the clinical diagnostic performance of Xpert Xpress SARS-CoV-2/Flu/RSV.

2. Methods

From May 2022 to April 2023, nasopharyngeal swabs from patients presenting to the hospital with fever were prospectively collected in Weifang Second People's Hospital. After routine testing of the samples, the remaining samples were collected and stored at -80℃. A total of 60 samples were collected, including 18 COVID-19 positive samples, influenza A positive samples, influenza B positive samples, 7 respiratory syncytial virus positive samples, and 11 samples which were negative for all four viruses. The kappa consistency test was used to determine the consistency of the results from the two test assays, and the cyclic threshold (Ct value) of positive samples was analyzed and compared by linear regression.

3. Results

Xpert Xpress SARS-CoV-2/Flu/RSV detected a total of 18 cases positive for COVID-19 with 100% sensitivity (18/18), 13 cases positive for influenza A with 100% sensitivity (13/13), 11 cases positive for influenza B with 100% sensitivity

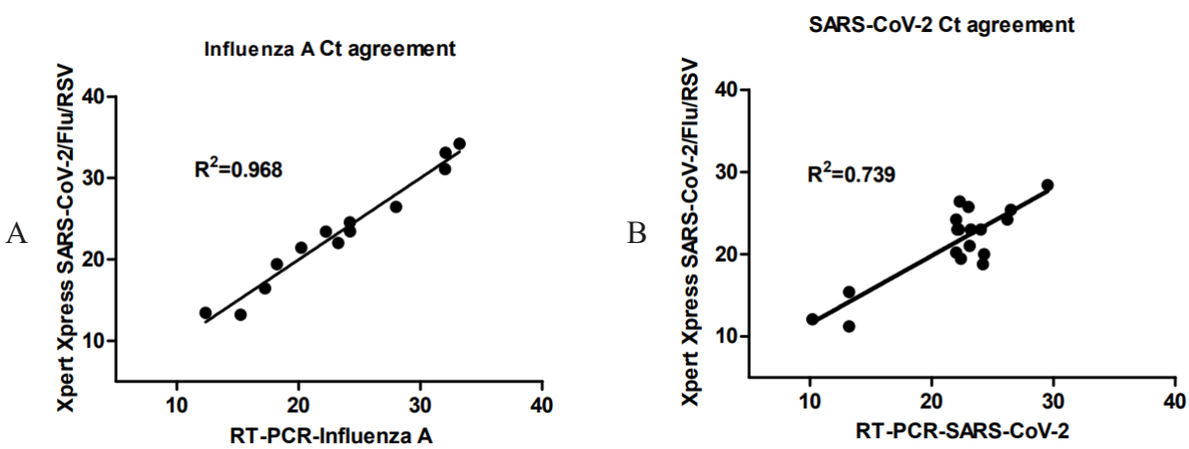
(11/11), and 7 cases positive for RSV (7/7; Table 1). The consistency of the two detection assayswas 100%. In the SARS-CoV-2/Flu/RSV test, SARS-CoV-2 can only be successfully amplified when both the E and N2 genes are detected on the same channel and cannot be read separately. ORF1ab gene and N gene were detected by RT-PCR. Therefore, the average Ct values for the two genes detected by RT-PCR were compared with the Ct values for SARS-CoV-2 amplification by the Xpert Xpress SARS-CoV-2/Flu/RSV assay. The Ct values reported by the two detection methods were highly consistent (Figure 1). The correlation between assays was lowest for SARS-CoV-2 (R2= 0.739), however for the influenza and RSV infections Ct values reported by the two detection methods were highly correlated: influenza A (R2= 0.968), influenza B (R2= 0.965) and RSV (R2=0.921). The lower correlation between assays for SARS-CoV2 may be because the target of the Daan assay SARS-CoV-2 amplification is ORF1ab and N gene, while the target of SARS-CoV-2 detected by the Xpert Xpress SARS-CoV-2/Flu/RSV assay is E gene and N gene.

Table 1. Xpert Xpress SARS-CoV-2 assay performance versus that of the NMPA-approved RT-PCR method

Classification		RT-PCR		Total	Sensitivity	Specificity	PPV[a]	NPV[b]	Kappa Value
					(% 95% CI)	(% 95% CI)	(% 95% CI)	(% 95% CI)	
		P	N						
Xpert Xpress SARS-CoV-2/Flu/RSV(SARS-CoV-2)	P	18	0	42	100(78.1-100)	100(89.6-100)	100(78.1-100)	100(89.6-100)	1
	N	0	42	42					
	Total	18	42	60					
Xpert Xpress SARS-CoV-2/Flu/RSV(FluA)	P	13	0	13	100(71.7-100)	100(90.6-100)	100(71.7-100)	100(90.6-100)	1
	N	0	47	47					
	Total	13	47	60					
Xpert Xpress SARS-CoV-2/Flu/RSV(FluB)	P	11	0	11	100(67.9-100)	100(90.9-100)	100(67.9-100)	100(90.9-100)	1
	N	0	49	49					
	Total	11	49	60					
Xpert Xpress SARS-CoV-2/Flu/RSV(RSV)	P	7	0	7	100(56.1-100)	100(91.6-100)	100(56.1-100)	100(91.6-100)	1
	N	0	53	53					
	Total	7	53	60					

[a]PPV: positive percent agreement.

[b]NPV: negative percent agreement.



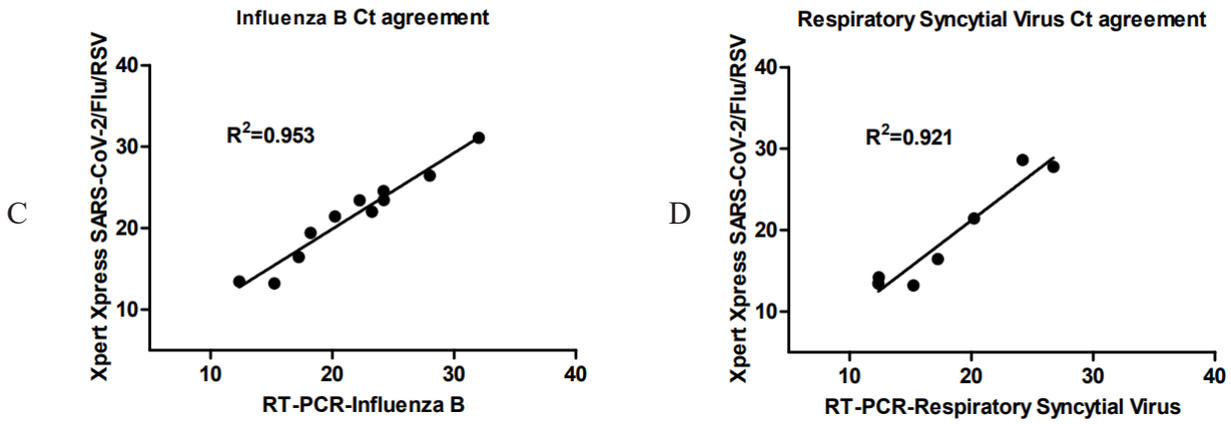


Figure 1. Correlation of the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test cycle threshold (Ct) values with the standard-of-care test Ct values for SARS-CoV-2 (A), influenza A virus (B), influenza B virus (C) and RSV (D).

4. Conclusions

Our investigation of the correlation between Xpert Xpress and Daan assays to rapidly and accurately distinguish between SARS-CoV2, influenza and RSV infection are consistent with those published previously[8]. To the best of our knowledge, this the first evaluation of the Xpert Xpress SARS-CoV-2 / Flu/RSV detection assay in clinical samples. Overall, both assays yielded nearly identical results for all targets, with nearly 100% agreement compared with the NMPA approved real-time RT-PCR assay. This indicates that the efficacy of the Xpert Xpress SARS-CoV-2/Flu/RSV assay is high, and that the assay is reproducible and rapid, particularly beneficial during the influenza season where hospital resources are under seasonal pressure. This assay is of great clinical value for distinguishing infections caused by these four viruses. Multi-center studies are needed to further verify the performance of the Xpert Xpress SARS-CoV-2/Flu/RSV assay kit.

Acknowledgments

The authors have no conflicts of interest to declare.

The project was approved by the Ethics Committee of the Weifang Second People's Hospital. The ethical approval reference number is ky2022-002-01. Prior to registration, each participant obtained written informed consent.

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