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Short communication

Evaluation of the respiratory NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and Alinity m Resp-4-Plex assays

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ABSTRACT

Background: December 2021 witnessed an unprecedented increase in SARS-CoV-2 infections in addition to the circulation of influenza A and respiratory syncytial viruses (RSV). Due to increased testing demands for SARS-CoV-2, influenza, and RSV associated with the overall increase in symptomatic respiratory infections, there is an urgent need for multiplex, automated, and high throughput assays in the diagnostic laboratories.
Methods: We compared the performance of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex to the standard of care influenza A, B, RSV, and SARS-CoV-2 assays used at the Johns Hopkins Microbiology Laboratory. A total of 181 remnant nasopharyngeal swab (NPS) specimens positive for influenza A ($n = 29$), influenza B ($n = 34$), RSV ($n = 40$), SARS-CoV-2 ($n = 33$), influenza A/RSV (n = 1), and negatives ($n = 44$) were tested by either or both assays.
Results: Both the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays showed 100% total agreement for all the tested analytes. For samples with available cycle threshold (Ct) values, comparable ranges were noted for all targets between the two assays and to the standard of care Ct values as well.
Conclusion: The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays showed high sensitivity and accuracy for all the analytes included in both tests. Implementing these assays will assist the diagnostic laboratories with the surge of testing during the 2021–2022 influenza season.

1. Introduction

The diagnostic laboratory testing of influenza and RSV increases during the seasons of their circulation. The COVID-19 pandemic impacted the circulation of respiratory viruses, including influenza and RSV, associated with measures of mitigating the spread of SARS-CoV-2 [1]. Upon relaxing the infection control measures, a rebound in respiratory viruses was notable after May 2021 with marked increases in enterovirus/rhinovirus and RSV [1]. The first detection of influenza in our system in 2021 was on October 27th followed by a slow increase in the number of cases to reach an average positivity of 3% in the month of December. The circulation of influenza in December correlated with a large increase in the circulation of SARS-CoV-2 that correlated with the introduction of the variant of concern Omicron [2]. This increase in symptomatic infections warranted an increase in the capacity of testing of SARS-CoV-2, influenza, and RSV to reveal the need for batched, fully automated, and multiplexed approaches for the three viruses under such circumstances. We hence evaluated the performance of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays compared to the standard of care methods for the detection and differentiation of influenza A, B, RSV, and SARS-CoV-2.

2. Materials and methods

2.1. Study site and ethics

The research was conducted with a waiver of consent under the Johns Hopkins IRB protocol IRB00246024. Remnant nasopharyngeal swabs (NPS) were collected after the standard of care diagnostic testing was performed.

2.2. Samples and standard of care testing

A total of 181 NPS specimens were randomly selected (based on the availability) from archived samples stored at −80 °C after the standard of care (SOC) testing for influenza A, influenza B, RSV, and/ or SARS-

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CoV-2. SOC testing included the Xpert® Xpress SARS-CoV-2/Flu/RSV ([3, 4]), Xpert Xpress Flu/RSV [5], Xpert Xpress SAR-CoV-2, the NxTAG Respiratory Pathogen Panel, NeuMoDx™ SARS-CoV-2, and Alinity m SARS-CoV-2. Influenza A samples had collection dates between January 2019 and February 2020, and influenza B samples were collected from April 2018 to February 2020. RSV samples were collected from January 2019 to January 2020. SARS-CoV-2 samples were collected from August 2021 to October 2021. Finally, negative samples were collected from March 2020 to October 2021.

2.3. Evaluated assays

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays use real-time PCR (RT-PCR) to detect and differentiate influenza A, influenza B, respiratory syncytial virus (RSV), and SARS-CoV-2. Both assays are performed on fully automated platforms; the NeuMoDx 288 system (Qiagen), and the Alinity m system (Abbott).

Table 1 summarizes the gene targets of the main evaluated and SOC assays. Notably, the two evaluated assays use one channel for detecting each virus and hence each virus has only one cycle threshold (Ct) value. Assays were run following the manufacturers' package inserts.

Table 1
Comparison of target genes of different assays used for the study.

	Influenza A	Influenza B	RSV	SARS-CoV-2	FDA EUA PI
Evaluated assays					
NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay	M	M	M	NSP2	https://www.fda.gov/media/147020/download
Alinity m Resp-4-Plex	M	Non-Structural 1	M	RdRp and N	https://www.fda.gov/media/146491/download
Standard of care assays					
Xpert Xpress SARS-CoV-2/Flu/RSV	PB2, PA, and M	M and NSP	nucleocapsid	N and E	https://www.fda.gov/media/142437/download
Xpert Xpress SARS-CoV-2				N and E	https://www.fda.gov/media/136314/download
Xpert Xpress Flu/RSV	PB2, PA, and M	M and NSP	nucleocapsid		
NeuMoDx SARS-CoV-2				NSP2 and N	https://www.fda.gov/media/136565/download
Alinity m SARS-CoV-2				RdRp and N	https://www.fda.gov/media/137979/download

M, Matrix; NSP, nonstructural protein; RdRp, RNA dependent RNA polymerase; N, Nucleocapsid; E, Envelope; PB, Polymerase basic protein; PA, Polymerase acidic.

3. Results

3.1. Agreement of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays with comparator standard-of-care methods

The SOC tests used for the diagnosis included the Xpert Xpress Flu/RSV (N = 105; 26 RSV, 26 influenza A, 29 influenza B, and 24 negatives), the Xpert Xpress SARS-CoV-2 (N = 22; 19 SARS-CoV-2, and 3 negatives), the Xpert® Xpress SARS-CoV-2/Flu/RSV (N = 12; 5 SARS-CoV-2, 7 negatives, and 1 RSV sample), the NxTAG Respiratory Pathogen Panel (N = 24; 14 RSV, 5 influenza A, and 5 influenza B), the NeuMoDx™ SARS-CoV-2 assay (N = 14; 9 SARS-CoV-2, and 5 negatives), and the Alinity m SARS-CoV-2 assay (N = 5; 5 negatives) (Table S1, and Table 2). Of the total samples, 83 samples were tested on the NeuMoDx™ FluA-B/RSV/SARS-CoV-2 Vantage assay (23 negatives, 14 SARS-CoV-2, 15 influenza B, 10 influenza A, and 21 RSV) and 30 samples were tested with the Alinity m Resp-4-Plex assay (10 negatives, 5 SARS-CoV-2, 5 influenza B, 5 influenza A, and 5 RSV). Additionally, 68 samples were tested both on the NeuMoDx™ FluA-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays (11 negatives, 14 SARS-CoV-2, 14 influenza B, 14 influenza A, 1 influenza A/RSV, and 14 RSV). Testing showed 100% total agreement for all targets tested with both assays (Table S1).

For samples with SOC Ct, we correlated the Cts for each target with Cts from the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays. Overall, excellent agreement for Ct values was notable between the two assays (Fig. 1). For SARS-CoV-2, an average increase of 0.69 Ct was noted with the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage compared to the SOC Ct values, however the Alinity m Resp-4-Plex assay showed an average of 1.71 Ct reduction (Table S1). For influenza A, the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage showed an average increase of 0.21 Ct and the Alinity m Resp-4-Plex showed an average of 0.4 Ct reduction. The Alinity m Resp-4-Plex and the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage showed an average increase of 0.74 and 1.03 Ct for influenza B respectively. The largest Ct difference was notable with RSV where both the Alinity m Resp-4-Plex and the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage assays showed a reduction of 4.02 and 4.16 Cts respectively (Table S1). Notably, the SOC assays use a different gene target for RSV (Table 1).

3.2. Reproducibility of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays

To assess the intra and inter-assay precision of both the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and the Alinity m Resp-4-Plex assays, clinical specimens positive for each target (influenza A, influenza B, RSV, SARS-CoV-2), and negatives were pooled and tested in replicates in the same run as well as over multiple days by different operators. An overall precision of 100% was noted for both assays for all tested analytes (Tables 3 and 4).

4. Discussion

The respiratory virus season of 2021–2022 has been particularly challenging due to the marked increase in symptomatic infections associated with the surge of SARS-CoV-2 positivity rates caused by the widespread prevalence of the Omicron variant [2]. The associated circulation of influenza viruses that started to increase in the beginning of December 2021 as well as RSV that showed a marked increase especially in July and August 2021 [1] led to an increase in the testing demand for SARS-CoV-2, influenza viruses, and RSV. Different commercial molecular test options that multiplexed influenza and RSV with SARS-CoV-2 have received the emergency use authorization (EUA) by the FDA. One of the very first small panels available was the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV which has been instrumental for testing

Table 2
Samples tested for each target and their original standard of care testing methods.

	Standard of care assays							Evaluated assays	
	Xpert Xpress Flu/RSV	Xpert Xpress SARS-CoV-2	Xpert Xpress SARS-CoV-2/ Flu/RSV	RPP (Luminex)	NeuMoDx SARS-CoV-2	Alinity SARS-CoV-2	Total samples Tested	NeuMoDx Flu A-B/ RSV/SARS-CoV-2 Vantage	Alinity m Resp-4-Plex
Influenza A	24	0	0	5	0	0	29	24	19
Influenza B	29	0	0	5	0	0	34	29	19
RSV	26	0	1*	14	0	0	40	35	19
Influenza A/ RSV	1	0	0	0	0	0	1	1	1
SARS-CoV-2	0	19	5	0	9	0	33	28	19
Negative	24	3	7	0	5	5	44	34	21
Total	104	22	12	24	14	5	181	151	98

* Sample was tested on two standard of care platforms but counted only once in total.

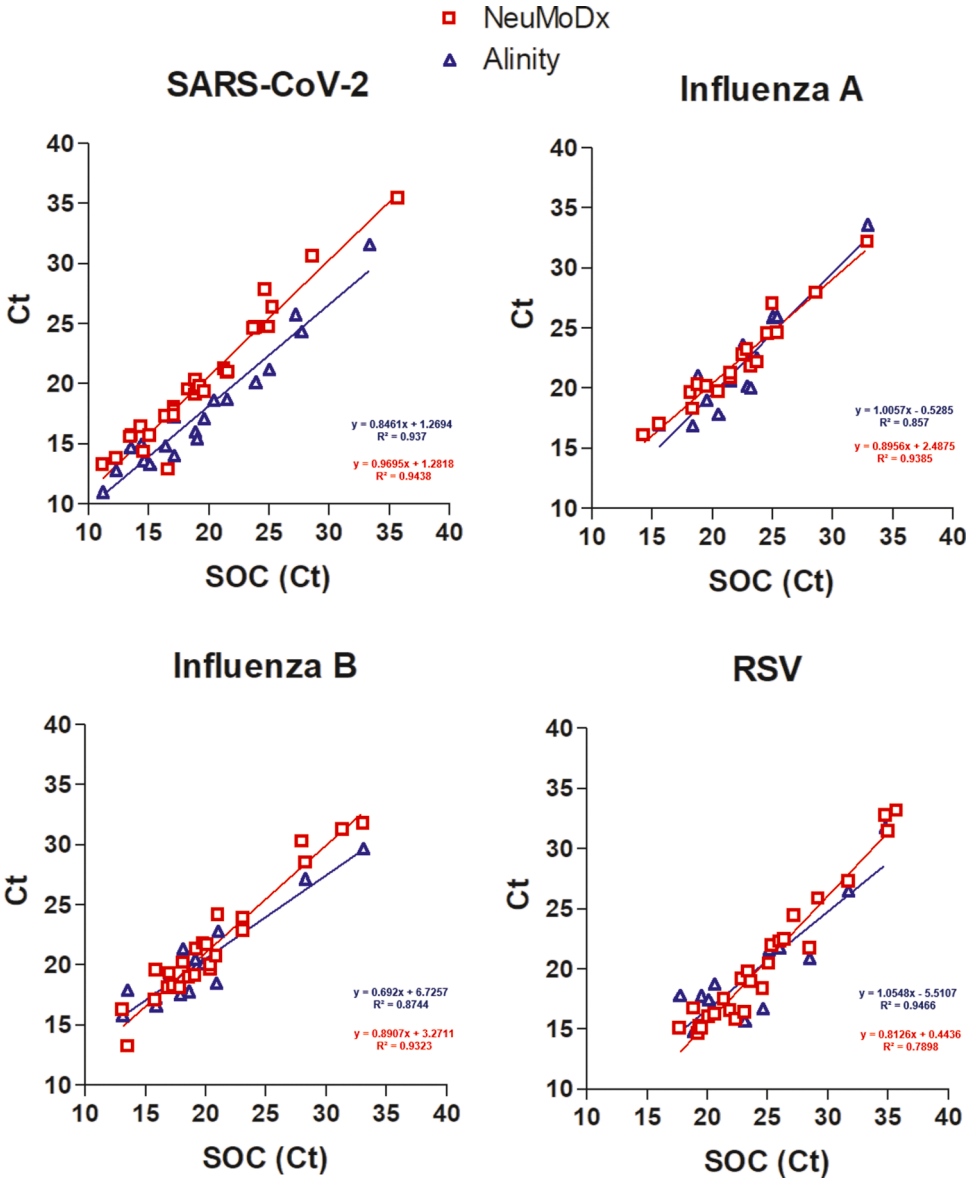

Fig. 1. Correlation of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage and Alinity m Resp-4-Plex assays cycle threshold (Ct) values with the standard of care (SOC) test Ct values for SARS-CoV-2, influenza A, influenza B, and RSV.

symptomatic patients with respiratory infections [3]. The capacity of testing using the Cepheid assay can be limited when unexpected surges challenge the large centralized diagnostic laboratories. Both the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 and Alinity m Resp-4-Plex are among only very few panel options that combined the testing of influenza A/B and RSV with SARS-CoV-2 and can be run in a fully automated, batched, and high throughput format.

The limitations of our report include using retrospective SOC results

Table 3
Reproducibility of the Alinity m Resp-4-Plex assays. Stdev, standard deviation; NEG, negative.

Influenza A	Ct		
Replicate 1	20.83	20.71	20.44
Replicate 2	20.14	21.02	20.35
Replicate 3	20.27	20.74	20.68
Stdev	0.30	0.14	0.14
Influenza B			
Replicate 1	19.64	19.49	19.43
Replicate 2	19.79	19.14	19.39
Replicate 3	19.32	19.58	19.39
Stdev	0.24	0.23	0.02
RSV			
Replicate 1	17.97	18.21	17.95
Replicate 2	18.13	17.51	17.88
Replicate 3	17.81	18.40	17.84
stdev	0.2	0.5	0.1
SARS-CoV-2			
Replicate 1	14.20	13.99	14.14
Replicate 2	14.40	13.95	14.31
Replicate 3	14.14	14.18	14.33
Stdev	0.14	0.12	0.10
Negative			
Replicate 1	NEG	NEG	NEG
Replicate 2	NEG	NEG	NEG
Replicate 3	NEG	NEG	NEG

Table 4
Reproducibility of the NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage. Stdev, standard deviation; NEG, negative.

Influenza A	Ct		
Replicate 1	21	21.26	21.34
Replicate 2	22.41	21.17	21.21
Replicate 3	20.93	20.97	21.2
Stdev	0.68	0.12	0.06
Influenza B			
Replicate 1	21.94	21.82	18.64
Replicate 2	21.47	20.36	21.3
Replicate 3	20.26	20.63	
stdev	0.87	0.78	1.88
RSV			
Replicate 1	19.1	19.53	18.83
Replicate 2	19.25	19.04	19.07
Replicate 3	18.84	19.59	18.52
stdev	0.2	0.3	0.3
SARS-CoV-2			
Replicate 1	18	17.53	19
Replicate 2	18.02	18.53	18.41
Replicate 3	17.87	18.03	18.59
Stdev	0.08	0.50	0.30
Negative			
Replicate 1	NEG	NEG	NEG
Replicate 2	NEG	NEG	NEG
Replicate 3	NEG	NEG	NEG

and archived frozen specimens. In addition, the Ct value comparisons might be biased by the gene targets used by each platform, the chemistry behind each technology, as well as the use of archived rather than fresh samples. The Ct analyses though showed the comparable trends of the two evaluated assays in different ranges of Ct values as well as to the standard of care methods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105164.

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