Performance Evaluation of a Multiplex SARS-CoV-2, Flu A/B, RSV RT-PCR Test for the Detection of COVID-19

Klaudyna Jacoby[1], Cameron May[1], Camilla Ulekleiv[2], Zoe Pounce[2], Géraldine Mercier[2], Peter Jacobs[2], Emily Hodgson[2], Darleen Welford[2], Jelena D. M. Feenstra[2], Manoj Gandhi[2], Andrzej Rutkowski[1]

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[1]Medicine Discovery Catapult Services, Alderley Park, Macclesfield, UK;
[2]Thermo Fisher Scientific, South San Francisco, CA, USA

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Background

Multiplex testing for SARS-CoV-2, Influenza and Respiratory Syncytial Virus (RSV) is recommended when these viruses are co-circulating in order to differentiate between the infections due to similar symptoms and also detect potential co-infections. Unlike winter 2020/2021, co-circulation of the three pathogens in the winter of 2021/2022 is anticipated. One of the concerns clinical laboratories face is whether implementation of multiplex tests for detection of all three pathogens will compromise the performance for SARS-CoV-2 detection. In the current study, we evaluated the clinical performance of the TaqPath™ COVID-19, Flu A/B, RSV Combo Kit in comparison to the widely used TaqPath™ COVID-19 CE-IVD RT-PCR kit for detection of SARS-CoV-2.

Methods

A retrospective study was performed on 430 (170 positive and 260 negative) leftover upper respiratory tract specimens collected in the UK from November 2020 to September 2021 and included different SARS-CoV-2 variants. SARS-CoV-2 status was previously characterized using the TaqPath™ COVID-19 CE-IVD RT-PCR kit. Following storage at -70ºC all samples were tested using the TaqPath™ COVID-19, Flu A/B, RSV Combo Kit and TaqPath™ COVID-19 CE-IVD RT-PCR Assay was repeated (Figure 1). Inconclusive results (N=2) were excluded from analysis and positive percent agreement (PPA) and negative percent agreement (NPA) for SARS-CoV-2 detection were calculated.

Figure 1. Study design

Results

SARS-CoV-2 was detected in 168 samples using the TaqPath™ COVID-19 CE-IVD RT-PCR Assay, and of those, 163 samples yielded a positive result with the TaqPath™ COVID-19, Flu A/B, RSV Combo Kit, resulting in a PPA of 97.02% (Table 1). For all 260 negative samples, a negative result was obtained using both assays, resulting in a NPA of 100% (Table 1). The Ct values of the positive cohort spanned the dynamic range of the assays with Ct<25 (40%), 25≤Ct<30 (34%); 30≤Ct<35 (20%); and Ct≥35 (6%) (Figure 2). All 5 SARS-CoV-2 discordant samples had a Ct>32.5 (Table 2).

Table 1. Concordance between TaqPath™ COVID-19, Flu A/B, RSV Combo Kit and TaqPath™ COVID-19 CE-IVD RT-PCR Kit

		TaqPath™ COVID-19 CE-IVD RT-PCR Kit		
		Positive	Negative	Total
TaqPath™ COVID-19, Flu A/B, RSV Combo Kit	Positive	163	0	163
	Negative	5	260	265
	Total	168	260	428
Positive Percent Agreement (95% CI)		**97.02%**	(93.22% to 98.72%)	
Negative Percent Agreement (95% CI)		**100.%**	(98.54% to 100%)	

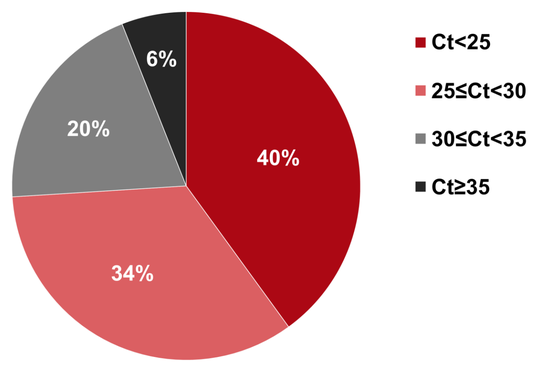

Figure 2. Distribution of Ct values for SARS-CoV-2 positive cohort

Table 2. Discordant samples mean Ct values

	TaqPath™ COVID-19 CE-IVD RT-PCR Kit		TaqPath™ COVID-19 FluA/B, RSV Combo Kit
Sample number	**Mean Ct value***	**Result**	**Result**
1	35.65	Positive	Negative
2	32.91	Positive	Negative
3	35.54	Positive	Negative
4	32.54	Positive	Negative
5	34.44	Positive	Negative

**TaqPath COVID-19 CE-IVD RT-PCR kit is a multiplex kit that produces a Ct value for each of the 3 targets: Orf1ab, N and S gene*

Conclusion

The TaqPath™ COVID-19, Flu A/B, RSV Combo Kit is a highly sensitive and specific assay for SARS-CoV-2 detection and offers comparable performance to the COVID-19-only testing. Multiplex RT-PCR testing for respiratory pathogens enables detection and differentiation between SARS-CoV-2, flu A/B and RSV infections along with the possibility to detect co-infections during the winter period when these viruses are co-circulating.

CE-IVD In Vitro Diagnostic Use