Head-to-Head Comparison of Two Respiratory Virus Research Assays

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Introduction

The persistence of circulating SARS-CoV-2 in conjunction with common seasonal respiratory viruses that share symptoms underscores the importance to monitor, detect, and differentiate SARS-CoV-2 from common causes of respiratory illnesses such as Influenza (Flu A and Flu B) and Respiratory Syncytial Virus (RSV). The goal of this study is to compare two research tools that can be used for this purpose, the ChromaCode HDPCR™ RV6 RUO Assay and the ThermoFisher TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay. The comparison includes analytical sensitivity, concordance across a set of 95 respiratory samples, ease of use/hands-on time, and time to result.

Samples

A combination of natural and contrived samples was used to compare the ChromaCode HDPCR™ RV6 RUO Assay and the ThermoFisher TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex assay. Samples were contrived using viral stocks (Zeptometrix, Table 1) with negative matrix as the diluent. The negative matrix. was a pool of nasopharyngeal samples collected in UTM and screened negative for all targets on each panel using either the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV or the Biofire® Respiratory Panel 2. For respiratory sample evaluation, a cohort of nasopharyngeal samples collected in UTM and originally tested on either the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV or the Biofire® Respiratory Panel 2 was used. Twenty (20) samples positive for each SARS-CoV-2, Influenza A, and RSV, 15 positive samples for Influenza B, and 20 negatives were run on each assay to evaluate performance.

Strain	PN	Target
A/Wisconsin/67/05	0810252CF	H3N2
B/Brisbane/33/08	0810253CF	FluB
CH93(18)-18	0810040CF	RSV B
2019-nCoV/USA-WA1/2020	0810587CFHI	SARS-CoV-2

Table 1. Zeptometrix strains used for contriving samples in this evaluation

The HDPCR RV6 RUO Assay is for research use only, not for use in diagnostic procedures.

Methods

The ThermoFisher TaqMan SARS-CoV-2, Flu A/B, RSV Multiplex assay and the ChromaCode HDPCR RV6 RUO assay were both used in accordance with their instructions for use. Prior to performing studies with the ThermoFisher assay, Ct cut-off values were established for downstream data analysis. A limit of detection (LoD) comparison using contrived samples was conducted at the dilution series seen in Table 2. Additionally, a concordance study between the assays using residual nasopharyngeal respiratory samples was performed. Turnaround time and hands on time for each assay were recorded by the operator.

Final Concentration (TCID 50/mL)

	Influenza A	Influenza B	RSV	SARS-CoV-2
Dilution A	1.10E+01	1.10E+00	3.00E-01	1.00E+01
Dilution B	1.10E+00	1.10E-01	3.00E-02	1.00E+00
Dilution C	1.10E-01	1.10E-02	3.00E-03	1.00E-01
Dilution D	1.10E-02	1.10E-03	3.00E-01	1.00E-02
Dilution E	1.10E-03	1.10E-04	3.00E-05	1.00E-03

Table 2. Limit of Detection Concentrations

Results

The ChromaCode assay had an approximate LoD value 10-fold more sensitive than the ThermoFisher assay for Flu A and value 100-fold more sensitive for Flu B. The assays had comparable LoD values for SARS-CoV-2 and RSV as seen in Table 3. The assays showed a per target concordance of 100% for Flu A, 98.9% for Flu B, 98.9% for RSV and 98.9% for SARS-CoV-2 across a set of 95 residual respiratory samples, yielding an overall 99.2% concordance of calls between the assays as illustrated in Table 4. Table 5 captures the timing of each assay step and shows the ThermoFisher assay had an overall shorter time to result by 17 minutes gained by shorter thermal cycling time. The ChromaCode assay, however, provided a 24-minute hands-on time savings gained by the streamlined analysis method with ChromaCode Cloud™.

	Flu A		Flu B		RSV		SARS CoV-2	
	ChromaCode	TaqMan	ChromaCode	TaqMan	ChromaCode	TaqMan	ChromaCode	TaqMan
Dilution A	100%	100%	100%	100%	100%	66%	100%	100%
	3/3	3/3	3/3	3/3	3/3	2/3	3/3	3/3
Dilution B	100%	100%	100%	0%	100%	100%	100%	100%
	3/3	3/3	3/3	0/3	3/3	3/3	3/3	3/3
Dilution C	100%	0%	100%	0%	100%	100%	66%	50%
	3/3	0/3	3/3	0/3	3/3	3/3	2/3	1/2*
Dilution D	0%	0%	33%	0%	100%	100%	0%	0%
	0/3	0/3	1/3	0/3	3/3	3/3	0/2*	0/2*
Dilution E	0%	0%	0%	0%	66%	66%	0%	33%
	0/3	0/3	0/3	0/3	2/3	2/3	0/3	1/3

* Invalid well excluded

Table 3. Limit of Detection performance comparison between both assays

Influenza A		TaqMan*		Influenza B		TaqMan*	
		+	-			+	-
HDPCR	+	20	0	HDPCR	+	15	1
	-	0	75		-	0	79

RSV A/B		TaqMan		SARS-CoV-2		TaqMan	
		+	-			+	-
HDPCR	+	23	1	HDPCR	+	20	0
	-	0	71		-	1	74

* TaqMan Assay reports Influenza A and Influenza B as a single Influenza target

Table 4. Performance comparison of natural samples between the two assays.

Steps	TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay	HDPCR™ Respiratory Virus 6 Assay
Extraction		
General Setup	32	32
Sample Setup	35	31
KingFisher	*24*	*24*
PCR		
Plate Setup	40	38
Run Setup	12	10
Cycling	*87*	*127*
Plate Import	15	15
Determine Threshold Settings	25	NA
Setup Cloud and Upload	NA	12
Analysis*	23	20
Total Time in Minutes	293	309
Total Instrument Time in Minutes	*111*	*151*
Hands on Time in Minutes	**182**	**158**

* Analysis time included incorporation of data into a table for comparison of results. PDF and .CSV reports automatically generated from ChromaCode Cloud.

Table 5. Turn Around and Hands On Time comparison of assays.

Conclusion

There are a multitude of research tools available for distinguishing between common respiratory illnesses; the goal of the study was to compare two options that utilize an open qPCR platform approach. The ThermoFisher and ChromaCode assays evaluated herein showed comparable performance with a few differences in time to result and user experience. The ChromaCode assay had an overall slightly longer time to result but had an improved user experience with a fully automated analysis application with embedded QC and reporting. Additionally, the ChromaCode assay benefitted from fewer set up steps, lower required volume of eluate, and discrimination between Influenza A and Influenza B. For research and epidemiological tracking, the differentiation of Influenzas A and B is essential, and is being reported at a national level by the United States Centers for Disease Control. The ChromaCode HDPCR RV6 RUO Assay offers a flexible and cost-effective solution for respiratory research needs.