Head to Head Comparison: HDPCR™ RV6 RUO Assay and ThermoFisher TaqMan® SARS-CoV-2, Flu A/B, RSV RUO Assay

Introduction

As waves of SARS-CoV-2 variants sweep across the globe, vaccinations and boosters are disseminated, antiviral treatments are released, and quarantine and masking mandates become localized, the future of COVID remains uncertain. The potential for total elimination of SARS-CoV-2 is predicted to be low based on the rate of mutation and the varying transmissibility of variants. Antigenic changes of variants may also affect vaccine protection.[1] Moreover, recent modeling suggests that variants will continue to emerge and have the potential to cause outbreaks,[2] indicating, at minimum, a short-term need for continuation of SARS-CoV-2 testing. Additionally, it is a widely held belief among researchers and clinicians that SARS-CoV-2 will become an endemic virus.[3] This transition from pandemic to endemic, in the presence of other respiratory viruses, will need to be continually monitored as more becomes understood about the SARS-CoV-2 virus. Research assays, to help research laboratories distinguish amongst common respiratory viruses, that are flexible, cost-effective, and easy to use and interpret will remain essential as SARS-CoV-2 transitions into an endemic virus.

This white paper compares the performance of the new ChromaCode HDPCR™ RV6 RUO Assay to the ThermoFisher TaqMan® SARS-CoV-2, Flu A/B, RSV RUO Assay. A general performance comparison, including limit of detection, time to result, and testing a cohort of residual respiratory samples was conducted, and is presented herein. The ChromaCode HDPCR RV6 RUO Assay introduces a research tool that will be indispensable as we look to the future. The ability for this assay to batch samples on a 96- or 384-well qPCR instrument, combined with the use of a standard workflow and easy to use interpretation software, allow for a simple solution in complicated times. The ChromaCode assay offers an overall comparable performance profile to the ThermoFisher assay, while being complete with identification of Influenza A separately from Influenza B, automated analysis software, and a simplified workflow.

Methods

Materials and Methods Overview

The HDPCR RV6 RUO Assay and the ThermoFisher TaqMan SARS-CoV-2, Flu A/B, RSV RT-PCR Assay were evaluated with samples that were extracted using the Thermo Scientific™ KingFisher™ Flex Extraction System and the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Thermo Scientific). All extractions utilized 200 µL input volume and were eluted into a final volume of 50 µL. Samples that were processed on the ThermoFisher assay used 190 µL of sample and were spiked with 10 µL of Sample Processing Control. Samples that were processed on the ChromaCode assay used 200 µL of sample neat into the extraction. Thermal cycling was run on an Applied Biosystems™ QuantStudio™ 5 384-well qPCR instrument. Data analysis was performed using the ChromaCode Cloud™ or the QuantStudio™ Design and Analysis software for the ChromaCode and ThermoFisher assays, respectively. Studies were performed by Arete Biosciences, LLC (Carlsbad).

Samples

Threshold setting (ThermoFisher assay only) and limit of detection studies were performed using contrived samples. The 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.

Table 2. Zeptometrix Strains Utilized in Study

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

ThermoFisher Assay Threshold Setting

Prior to performing studies with the ThermoFisher assay, Ct cut-off values were established for downstream data analysis. Ct values in each reporting channel were determined by the user following the assay instructions for use.[4] A 5-part, 10-fold dilution series was run for each target, along with a representative low, medium, and high concentration sample for each analyte and 3 negatives. Representative samples, used to ensure thresholds set were appropriately, were chosen from available cohort of natural samples. Low, medium, and high concentrations were based on Ct values reported from original method of characterization.

Limit of Detection

The limit of detection for both assays was compared using a 5-part, 10-fold dilution series. Concentrations for each target are shown in Table 2. Three unique extraction replicates were run for each concentration on each assay.

Table 1. Limit of Detection Target Concentrations

	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

Respiratory Sample Evaluation

Residual respiratory samples were evaluated on both assays according to assay instructions for use.[4,5]

Assay Timing

Times were recorded using a laboratory timer for each step of each assay to compare overall time to result and workflow steps of each assay.

Results

ThermoFisher Assay Ct Validation

The thresholding values were set where signals started into the exponential phase of amplification above the background signals. The settings determined for the assay during validation are shown in Table 3. These were verified against the known samples run during the validation activity.

Table 3. ThermoFisher Assay Validated Ct Cut-offs

Reporter	FAM	VIC	ABY	JUN
Threshold (RFU)	27,500	12,000	5,000	10,000
Baseline Start Cycle	5	5	10	5
Baseline End	auto	auto	auto	auto

Limit of Detection

A series of five 10-fold dilutions were run on both the ChromaCode and ThermoFisher assays to compare relative LoD levels between the assays. The results are shown in Table 4. The ChromaCode assay had an approximate LoD value 10-fold lower than the ThermoFisher assay for Influenza A and 100-fold lower for Influenza B. Furthermore, the ChromaCode assay discriminates between Influenza A and

Influenza B, while the ThermoFisher assay reports as Influenza A/B. Both assays had comparable performance for SARS-CoV-2 and RSV.

Respiratory Sample Evaluation

The residual respiratory sample evaluation results for each assay in comparison to each other are presented in Table 5. 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.

Assay Timing

The timing of steps of both the ThermoFisher and ChromaCode assay are recorded in Table 6. The assays overall had a comparable overall run time, with ThermoFisher's assay slightly shorter (17 minutes). The thermal cycling for the ThermoFisher assay was substantially shorter than that of the ChromaCode assay, whereas automated analysis and set up saved time on the ChromaCode assay, giving the ChromaCode assay a 24-minute reduced hands-on time.

Table 4. Limit of Detection Comparison

	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 5. Head to Head Residual Sample Comparison

Influenza A		TaqMan*	
		+	-
HDPCR	+	20	0
	-	0	75

Influenza B		TaqMan*	
		+	-
HDPCR	+	15	1
	-	0	79

RSV A/B		TaqMan	
		+	-
HDPCR	+	23	1
	-	0	71

SARS-CoV-2		TaqMan	
		+	-
HDPCR	+	20	0
	-	1	74

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

Table 6. Assay Time Comparison in Minutes

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.

Discussion

The two assays tested herein were compared in their limit of detection, as well as performance using a set of residual respiratory samples. The ChromaCode assay showed a lower limit of detection for the influenza targets in comparison to the ThermoFisher assay. The additional targets showed similar results in the limit of detection comparison. Both research use only assays performed with a high degree of agreement to each other with an overall 99.2% concordance of calls between the assays. The TaqMan assay called a single SARS-CoV-2 presence that was not seen in the ChromaCode assay, whereas the ChromaCode assay called a single RSV and Influenza B that were not seen in the TaqMan assay. The overall time to results was slightly faster with the ThermoFisher assay, but much of the time savings

came in thermal cycling time, while the ChromaCode assay showed an overall lower hands-on time, saving 24-minutes of technician time. The ChromaCode assay allowed for time savings in assay set up and streamlined data analysis with zero on-boarding time using the ChromaCode Cloud. The ChromaCode Cloud offers intuitive visualization of data that is accessible from anywhere you can access the internet. The ChromaCode Cloud houses all data analysis algorithms and any necessary thresholds for a ChromaCode assay, thereby helping to ensure consistency amongst users.

An additional difference to highlight is the volume of eluate used for each assay. The ThermoFisher assay uses 17.5 µL of the eluate from a KingFisher run, whereas the ChromaCode assay uses 5 µL. The larger amount of eluate used in the ThermoFisher assay makes re-runs challenging, especially if using a multichannel or an automation platform. The larger amount of runs that can be gained from a single extraction from the ChromaCode assay may allow for greater flexibility in a wider variety of applications.

Each assay leveraged a different control strategy. ThermoFisher recommended positive and negative run controls, but neither controlled for the extraction process, whereas the positive and negative run controls for ChromaCode underwent extraction. The ChromaCode assay utilized human RNase P as a dual sample integrity and process control, whereas the ThermoFisher assay spiked in an exogenous sample processing control. The addition of an exogenous sample processing control mandates an additional pipetting step that gives opportunity to introduce pipetting errors and gives no indication of the integrity of the sample. In this study, there were 4 invalid samples seen in the ThermoFisher assay and 1 invalid sample seen in the ChromaCode assay due to failure to amplify any targets.

Finally, for use in surveillance and epidemiological studies, the ChromaCode assay has an increased utility based on its ability to distinguish between Influenza A and Influenza B. International programs like the World Health Organization's Global Influenza Surveillance and Response System receive data on Influenza types from all over the world with Influenza A and Influenza B reported separately. Additionally, the potential differences in outcomes and differences in responses to treatments between infections from Influenza A and B are still being actively investigated in the literature.[6] Being able to distinguish between Influenza A and Influenza B is of great value in many current research applications.

Conclusions

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. The ChromaCode HDPCR RV6 RUO assay offers a flexible and cost-effective solution for respiratory research needs.

References

1. Monto, AS. The Future of SARS-CoV-2 Vaccination – Lessons from Influenza. New England Journal of Medicine. 2021; 385:1825-1827.
2. Dyson, L., Hill, E.M., Moore, S. *et al.* Possible future waves of SARS-CoV-2 infection generated by variants of concern with a range of characteristics. Nat Communications. 2021;12:5730.
3. Phillips N. The coronavirus is here to stay - here's what that means. Nature. 2021;590:382-4.
4. TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay Quick Reference Guide. Pub. No. MAN0019614 Rev. B.0
5. ChromaCode HDPCR™ RV6 RUO Instructions for Use. DOCID 582237.1
6. Hassan Zaraket, Aeron C. Hurt, Barry Clinch, Ian Barr, Nelson Lee. Burden of influenza B virus infection and considerations for clinical management, Antiviral Research. 2021;185:104970.