Evaluation of the ChromaCode HDPCR™ RV6 RUO Assay

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Background

In December of 2019, an outbreak of pneumonia was reported to the World Health Organization. The etiology of this outbreak was identified as novel coronavirus (SARS-CoV-2), which has resulted in a global pandemic. The persistence of circulating SARS-CoV-2 leads to the need for a test that can detect and differentiate SARS-CoV-2 from other common causes of respiratory illnesses such as influenza and respiratory syncytial virus (RSV). ChromaCode's High Definition PCR (HDPCR™)[1,2] RV6 RUO assay is a real time PCR test that provides qualitative detection of SARS-CoV-2, Influenza A, Influenza B, and RSV A/B from upper respiratory specimens. An evaluation in an infectious disease molecular laboratory was performed and presented herein.

Methods

A combination of residual and contrived samples were used to evaluate the ChromaCode HDPCR™ RV6 RUO assay. Residual upper respiratory samples tested with the standard of care were enrolled in this study to evaluate the RV6 RUO assay. A total of 201 samples originally tested using the standard of care (a combination of Diasorin Simplexa®, TaqPath™, and Genmark ePlex® tests) were enrolled: 17 assisted mid-turbinate, 15 contrived Flu B samples, and 179 nasopharyngeal swabs (NPS). The contrived samples were made utilizing dilutions of Zeptometrix Influenza B material (PN 0810253CF LN 325137) in negative NPS matrix to achieve final concentrations of 0.02-0.04 TCID50/mL. All samples were extracted on a KingFisher and run on an ABI 7500 Fast Dx.

References

1. Rajagopal, A., Yurk, D., Shin, C. et al. Significant Expansion of Real-Time PCR Multiplexing with Traditional Chemistries using Amplitude Modulation. Sci Rep 9, 1053 (2019).
2. Jacky, Lucien, et al. "Robust Multichannel Encoding for Highly Multiplexed Quantitative PCR." Analytical Chemistry 93.9 (2021): 4208-4216.

Results

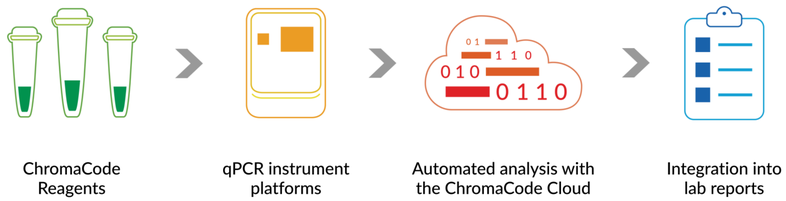

Figure 1. Outline of the ChromaCode HDPCR workflow, which seamlessly integrates into routine laboratory qPCR testing.

A minimum of 20 positive specimens were run for all four of the targets on the RV6 RUO assay, Influenza A, Influenza B, SARS-CoV-2, and RSV A/B. All enrolled specimens were run through the workflow seen in Figure 1, where HDPCR reagents are leveraged to create a mastermix, which can be run on various qPCR instruments, and results are interpreted using ChromaCode Cloud™ software. The software automatically analyzes the data based on end point fluorescence values established by calibrators run on the plate and yields results, as seen in Figure 2.

Figure 3 summarizes the data collected in the study from all 201 specimens. The HDPCR RV6 RUO assay and the standard of care Diasorin assays were 99% concordant for the Influenza A target calls, 100% concordant for the Influenza B calls, 97.5% concordant for the RSV calls, and 99.5% concordant for the SARS-CoV-2 calls. There was no identified higher rate of discrepancy among the mid-turbinate specimens versus the NPS specimens.

Figure 2. ChromaCode Cloud deconvolutes data and makes calls across the four utilized channels on the qPCR instrument for all samples automatically.

Influenza A		UCLA SoC	
		+	-
HDPCR	+	21	1
	-	1	178

99.0% Concordance Flu A

Influenza B		UCLA SoC	
		+	-
HDPCR	+	20	0
	-	0	181

100% Concordance Flu B

RSV A/B		UCLA SoC	
		+	-
HDPCR	+	37	3
	-	2	159

97.5% Concordance RSV

SARS-CoV-2		UCLA SoC	
		+	-
HDPCR	+	39	1
	-	0	161

99.5% Concordance SARS-CoV-2

Figure 3. The concordance by target compared to the standard of care method.

Discussions and Conclusions

Of the 201 samples enrolled in this study, the ChromaCode HDPCR RV6 RUO Assay performed with 99% concordance to the Diasorin Simplexa tests that are current standard of care at UCLA. In this study, 7 of the samples were assisted mid-turbinate and all of these 7 samples were concordant between the RV6 RUO Assay and the Standard of Care. The compatibility with multiple sample types is a feature that can be further explored about the RV6 RUO Assay in various applications. As a result of the COVID pandemic, more people have been exposed to different sampling methodologies for identifying respiratory illnesses and may now desire a less invasive sample type if available. An additional potential use case for the RV6 RUO Assay is to leverage the flexible reporting feature in the ChromaCode Cloud Software that automatically generates a report from raw data. Flexible reporting allows for any subset of targets to reported out from the assay, rather than all the targets. This could be configured to report out only for the COVID target or the full panel depending on time of the year, for example.
ChromaCode's HDPCR RV6 RUO Assay illustrates an example of a cost effective and flexible research tool that performed comparably to the current UCLA standard of care. Additionally, all targets were covered with a single assay, rather than a combination of different assays, streamlining workflows and maximizing efficiencies.

Limitations

One important piece of this study that is currently missing is the resolution of the discrepant results leveraging a validated comparator method. Currently, Sanger sequencing based methods are being validated to resolve the discrepant results that were seen on this assay. That will allow for a final arbitrator for all these specimens and complete the comparison of the assays.
Additionally, the majority of these samples were collected in the winter of 2021/2022, which represented a season with very little Influenza B. Due to this scarcity, 15 Influenza B samples were contrived from control material in negative NPS matrix to yield a cohort of 20 samples positive for Influenza B. The samples were contrived to be about 2X higher than the limit of detection of the RV6 RUO assay as illustrated in the instructions for use.

Future Directions

This study enrolled all specimens that were run during a set period in the winter of 2021/2022. The cohort included 17 assisted mid-turbinate specimens out of 201 total specimens. One driver of this research study was to start to gauge compatibility of a multi-analyte test with various sample types beyond NPS. A larger cohort of varied upper respiratory specimens with a broad representation of positives for all targets would be necessary to further expand this study.
An additional data point that could be analyzed during this expansion would be the number of times that different upper respiratory sample types were ordered for various suspected illnesses. An expansion to this study with both technical compatibility and analysis of percentages of sample types ordered would be of great value.