Development of a high-throughput multiplex Research Use Only (RUO) assay for the simultaneous identification and differentiation of viral RNA from SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV)

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BACKGROUND

The persistence of circulating SARS-CoV-2 leads to the need for a research tool that can further help in understanding viral prevalence trends and differentiate viral RNA of SARS-CoV-2 from other common respiratory illness such as Influenza and Respiratory Syncytial Virus (RSV). ChromaCode Inc. is developing a high-throughput HDPCR™ RV6 RUO Assay for Research Use Only (RUO), not for use in diagnostic procedures, that simultaneously identifies and differentiates viral RNA from SARS-CoV-2, Influenza A, Influenza B, and RSV A/B. The inclusive design of the assay reports 4 viral targets, human RNase P, to ensure sample integrity, and an optional sample processing control. Influenza A interrogates for H1, H1N1, and H3 and RSV interrogates for RSV A and RSV B. Therefore, these common respiratory viral targets can be differentiated with one sample and one assay, while ensuring sample integrity throughout the process. The HDPCR RV6 RUO Assay was designed for use on both 96-well and 384 well qPCR instruments, enabling research laboratories to scale and maximize their efforts using their existing instrumentation and footprint.

PROTOCOL & ANALYSIS

The HDPCR RV6 RUO Assay is a real-time reverse transcription polymerase chain reaction (qRT-PCR) test that employs TaqMan® chemistry and ChromaCode Cloud™ to detect and report multiple targets in a single-color channel. The RV6 primer and probe sets are designed to detect, with high inclusivity, viral RNA from Influenza A, Influenza B, Respiratory Syncytial Virus A and B, and SARS-CoV-2. RV6 uses multiple reporter and dye quencher sets to consolidate these detections into a single well.

MATERIALS & METHODS

The RV6 Assay was evaluated on a total of 5 qPCR instruments from Thermo Scientific™. All samples were processed using the Thermo Scientific KingFisher™ Flex extraction platform using 180 µL of sample, with the addition of 20 µL of sample processing control and eluting into a final volume of 50 µL with MVP II kits. All data were processed on the ChromaCode Cloud.

LIMIT OF DETECTION

The RV6 Assay Limit of Detection (LoD) study was conducted using a two-stage approach. Concentrations of 8 viral strains purchased from Zeptometrix (3 Influenza A, 2 Influenza B, 2 RSV and 1 SARS-CoV-2) were analyzed to determine the lowest concentration of a single analyte that the RV6 Assay could detect in ≥19/20 replicates. LoD studies were conducted on the Applied Biosystems™ 7500 Fast Dx.

Reportable	Strain or Isolate	Results
Influenza A	Influenza A H3N2 (A/Perth/16/2009)	0.01 $TCID_{50}$/mL
	Influenza A H1N1 (A/New Caledonia/20/1999)	0.11 $TCID_{50}$/mL
	Influenza A H1N1 2009 (A/NY/01/09)	0.33 $TCID_{50}$/mL
Influenza B	Influenza B (B/Brisbane/60/2008)	0.01 $TCID_{50}$/mL
	Influenza B Yamagata_Florida/04/06	0.03 $TCID_{50}$/mL
RSV	Respiratory Syncytial Virus A (2006 Isolate)	0.33 $TCID_{50}$/mL
	Respiratory Syncytial Virus B CH93(18)-18	0.03 $TCID_{50}$/mL
SARS-CoV-2	2019-nCoV/USA-WA1/2020	10.0 $TCID_{50}$/mL

Table 1. Limit of Detection for HDPCR RV6 RUO Assay
Results of Stage 2 confirmation of LoD where minimum of 19/20 replicates were correctly detected using RV6 RUO Assay.

INSTRUMENT COMPATIBILITY

Instrument compatibility for the RV6 RUO Assay was confirmed by evaluating 4 concentrations (3x, 2x, 1.5x, 1x, and 0.5x LoD) for each target in the same two-staged approach as utilized when determining the LoD on the 7500 Fast Dx. The results from this study determined that all instruments evaluated (7500 Fast, QuantStudio 7 (0.1mL, 96 well), QuantStudio 5 (0.2mL, 96 well), QuantStudio 5 (384 well) and QuantStudio 12K (384-well) are equivalent in the RV6 Assay based on the acceptance criteria that all instruments are ≤ 3X away from the LoD established on the 7500 Fast Dx

STUDY EVALUATION UTILIZING RESPIRATORY SAMPLES

A study evaluation utilizing respiratory samples was performed using the RV6 RUO Assay. 250 residual nasopharyngeal swab samples (50 each target and 50 negative) collected in either UTM or VTM were analyzed utilizing the RV6 RUO Assay. Sample testing results were compared with previously characterized results from either the Cepheid Xpert® Xpress FluA/B/RSV assay or the Cepheid FluA/B/RSV/SARS-CoV-2.

Instrument	Target	Present Target/Total Tested (95% CI)	Absent Target/Total Tested (95% CI)
7500 Fast Dx	Influenza A	100.0% (91.1%-100%)	99.5% (96.8%-100%)
	Influenza B	100.0% (91.1%-100%)	100% (97.7%-100%)
	RSV	96.00% (85.1%, 99.3%)	100% (97.7%, 100%)
	SARS-CoV-2	98.00% (88.0%, 99.9%)	100% (97.7%, 100%)
QuantStudio 5	Influenza A	100.0% (91.1%, 100%)	100.0% (97.7%, 100%)
	Influenza B	100.0% (91.1%, 100%)	99.5% (96.8%, 100%)
	RSV	94.0% (82.5%, 98.4%)	100.0% (97.7%, 100%)
	SARS-CoV-2	94.0% (82.5%, 98.4%)	100.0% (97.7%, 100%)
QuantStudio 7	Influenza A	100.0% (91.1%, 100%)	100.0% (97.7%, 100%)
	Influenza B	100.0% (91.1%, 100%)	100.0% (97.7%, 100%)
	RSV	98.0% (88.0%, 99.9%)	100.0% (97.7%, 100%)
	SARS-CoV-2	96.0% (85.1%, 99.3%)	100.0% (97.7%, 100%)
QuantStudio 12K Flex	Influenza A	100.0% (91.1%, 100%)	99.0% (96.%, 99.8%)
	Influenza B	100.0% (91.1%, 100%)	100.0% (97.7%, 100%)
	RSV	94.0% (82.5%, 98.4%)	100.0% (97.7%, 100%)
	SARS-CoV-2	96.0% (85.1%, 99.3%)	100.0% (97.7%, 100%)

Table 2. Study Evaluation Utilizing Respiratory Samples
Confidence intervals were calculated using the score method with continuity correction.

CONCLUSION

ChromaCode's RV6 RUO Assay is a sensitive, scalable, and high-throughput solution for detection of common viral targets in respiratory samples. The RV6 RUO Assay shows great versatility amongst common qPCR platforms, is equipped with an endogenous sample integrity control, and streamlines data interpretation with use of ChromaCode Cloud.

[1] This protocol is not approved or cleared by the US FDA and is in development. For research use only, not for use in diagnostic procedures.