Expansion of ChromaCode's HDPCR™ SARS-CoV-2 Assay EUA to Increase Access and Throughput

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BACKGROUND

President Biden's National Strategy for the COVID-19 Response and Pandemic Preparedness includes expanded testing as part of the comprehensive effort to control the virus[1], driving the need for test adaptability for laboratories. In response, ChromaCode's high-definition polymerase chain reaction (HDPCR™) SARS-CoV-2 Assay real-time PCR instrument compatibility was expanded to eight (8) Applied Biosystems (ABI) instrument configurations, including 96 and 384 well formats. The HDPCR SARS-CoV-2 Assay is for use under the Food and Drug Administration's Emergency Use Authorization. The HDPCR SARS-CoV-2 Assay is a reverse transcription real-time polymerase chain reaction (qRT-PCR) test contained in a single reaction well. The test is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in human upper respiratory specimens taken from individuals exhibiting symptoms of infection and who are suspected of having COVID-19 by their healthcare provider.

MATERIALS

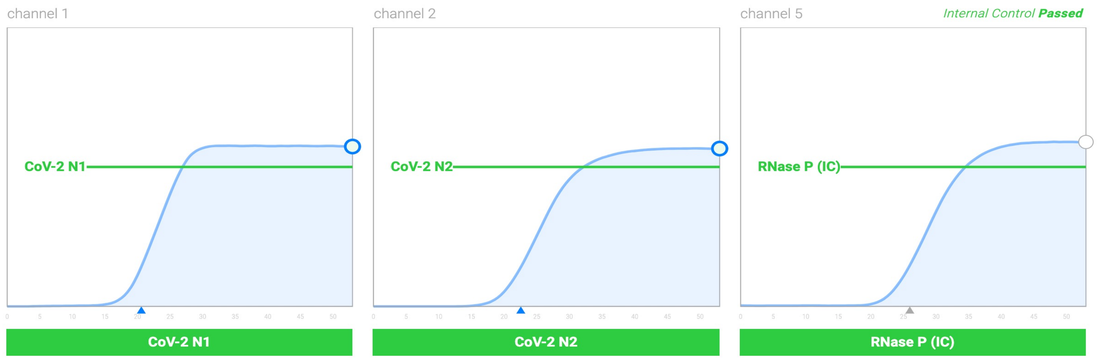
The Limit of Detection (LoD) was determined for each real-time PCR instrument/well format following a two-stage approach using dilutions of Armored RNA Quant® SARS-Cov-2 Control (Asuragen) spiked into residual negative NPS. All sample extractions used 200 µL input with elution to 50 µL, and 5 µL input to the assay. In the first stage, testing was performed across a range of input concentrations to estimate the LoD. The LoD was confirmed in the second stage with 20 replicates. Clinical performance was established for the 96 and 384 well configurations using positive and negative clinical specimens characterized with a highly sensitive SARS-CoV-2 EUA assay comparator method

[1] National Strategy for the COVID-19 Response and Pandemic Preparedness, January 2021. https://www.whitehouse.gov/wp-content/uploads/2021/01/National-Strategy-for-the-COVID-19-Response-and-Pandemic-Preparedness.pdf

WORKFLOW & ANALYSIS

The HDPCR SARS-CoV-2 Assay uses TaqMan® probe chemistry and proprietary analysis to allow qRT-PCR multiplexing within a single-well. Viral nucleic acid is extracted from human nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, and nasal wash using either the Roche MagNA Pure 24 or the Thermo Scientific KingFisher Flex. The product includes the same N1 and N2 oligonucleotide primer and probe sequences for the detection of the SARS-CoV-2 viral RNA and the human RNase P gene used in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel for Emergency Use Only. Alternate reporter and quencher dyes are used to consolidate the reaction into a single well. Additional materials in the HDPCR SARS-CoV-2 Assay include enzyme and buffer mixes, extraction and assay run controls, and calibrators to ensure accurate results.

Figure 1. HDPCR SARS-CoV-2 Assay, SARS-CoV-2 detected



CLINICAL EVALUATION

Clinical Evaluation of the HDPCR SARS-CoV-2 Assay was conducted on both the 384 and 96-Well format on the Applied Biosystems QuantStudio 7 instrument. The specimens were enrolled using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel with Promega Maxwell® extraction chemistry and run on the ABI QuantStudio Dx instrument. Specimens (NP swabs and combo NP/OP swabs collected in VTM) to be run on the HDPCR SARS-CoV-2 Assay were extracted using the Roche MagNA Pure 24 Pathogen 200 2.0 Protocol with the Total Nucleic Acid Isolation Kit.

Table 1. Clinical Evaluation QuantStudio 7, 384-Well Instrument

Comparator Result	Total	Correct	Incorrect	Invalid	Presumptive Positive	Percent Agreement	95% CI
Positive	67	60	5 (FN)	1	1	89.6%	79.6-95.7%
Negative	84	80	4 (FP)	0	0	95.2%	88.2-98.7%

Based on the comparator method Ct values used for the study, 3 of the 5 false negatives and the presumptive positive are samples near or below the LoD for the comparator assay.

Table 2. Clinical Evaluation QuantStudio 7, 96-Well Instrument

Comparator Result	Total	Correct	Incorrect	Invalid	Presumptive Positive	Percent Agreement	95% CI
Positive	39	37	2 (FN)	0	0	94.9 %	82.7 - 99.4%
Negative	41	39	2 (FP)	0	0	95.1 %	83.4 – 99.4%

[2]COVID-19 Emergency Use Authorization Only. For in vitro diagnostic (IVD) Use | Rx Only

[3]Review under the EUA program is pending. This configuration is being distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C

LIMIT OF DETECTION

The LoD of the HDPCR SARS-CoV-2 Assay was determined using a two-stage approach. In the first stage, a preliminary LoD was established by testing either 3 or 5 replicates at each selected serial dilution. The lowest concentrations to detect either 3/3 or 5/5 replicates were moved to further evaluation in Stage 2, where the LoD was confirmed on each instrument by testing 20 replicates. Both Stage 1 and Stage 2 LoD testing used Armored RNA Quant® SARS-CoV-2 control obtained from Asuragen (Catalog Number 52030) spiked into negative nasopharyngeal swab specimens at designated concentrations. The results of this Stage 2 confirmation are seen in Table 12, indicating the established LoD for each qPCR instrument tested.

Tables 4-5. ChromaCode HDPCR SARS-CoV-2 Assay LoD

Roche MagNa Pure 24A: Pathogen 200 2.0, Total Nucleic Acid Isolation Kit	
qPCR Instrument	**LoD, copies/mL**
QuantStudio 7, 96 Well[2]	1000
QuantStudio 7, 384 Well[3]	2000
7500 Fast Dx[3]	2000
7500 Fast[2]	1000

Thermo Scientific King Fisher: MVP_Flex Protocol, MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	
qPCR Instrument	**LoD, copies/mL**
QuantStudio 12K Flex, 96-Well[2]	250
QuantStudio 12K Flex, 384-Well[3]	1000
QuantStudio 5, 96-Well 0.2 mL[3]	1000
QuantStudio 5, 384-Well[3]	1000

CONCLUSION

ChromaCode's HDPCR SARS-CoV-2 Assay is a highly sensitive test compatible with eight (8) ABI real-time PCR Instruments in both 96 and 384 well formats, thereby enabling access to a wide number of laboratories. The inclusion of the 384 well format significantly increases testing capacity, allowing for high volume laboratories to maintain high sample throughput.