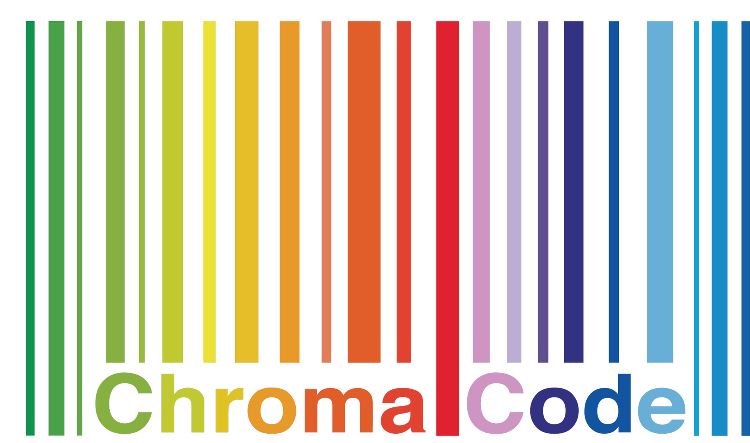

Alternative protocol for SARS-CoV-2 Testing Using a Heat Lysis Method for Respiratory Samples

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BACKGROUND

The SARS-CoV-2 pandemic has demonstrated the need for streamlined protocols with high-throughput testing. In extraction-based testing, limited extraction reagents and required proprietary instrumentation may pose a bottleneck for labs. As a potential solution, ChromaCode explored a Direct Extraction[1] research protocol for the HDPCR™ SARS-CoV-2 Assay. This is a research protocol extension of the HDPCR SARS-CoV-2 Assay and is not authorized, cleared, or approved. This protocol allows for the processing of specimens without an extraction system. In lieu of an extraction system, the Direct Extraction protocol utilizes heat lysis to release the viral nucleic acid, which is then directly added to the HDPCR master mix.

PROTOCOL & 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 heat lysed directly from human NPS, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, and nasal wash using the Direct Extraction protocol. This protocol enables users to perform sample preparation with heat lysis using standard lab equipment. The product assays for the N1 and N2 loci of the SARS-CoV-2 virus, alongside an endogenous human RNase P control to serve as an internal and sample integrity control. The HDPCR SARS-CoV-2 Assay allows for endpoint fluorescence based detection of the SARS-CoV-2 virus with a convenient heat lysis based protocol on existing qPCR instrumentation with efficient results reporting on the ChromaCode Cloud™.

MATERIALS & METHODS

The Limit of Detection (LoD) study, a respiratory sample evaluation study, and the effect of interfering substances on respiratory samples was determined for the Direct Extraction protocol. The LoD was established on 6 qPCR instruments with dilutions of gamma-irradiated SARS-CoV-2 virus from BEI, spiked into residual, negative nasopharyngeal swab (NPS) samples.. Performance on respiratory samples was assessed with 30 frozen, retrospective samples where SARS-CoV-2 was detected and 30 where it was not using the Direct Extraction protocol compared to an external Emergency Use Authorized (EUA) comparator assay (CDC 2019-nCoV) on three PCR platforms. The effect of 13 potential interfering substances was evaluated at 3X LoD using gamma-irradiated SARS-CoV-2 spiked into negative NPS.

LIMIT OF DETECTION

The LoD of the HDPCR SARS-CoV-2 Assay – Direct Extraction protocol was determined using a two-stage approach on 6 qPCR instruments. In the first stage, a preliminary LoD was established by testing 5 replicates at each selected serial dilution. The lowest concentrations to detect 4/5 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 gamma-irradiated SARS-CoV-2 spiked into pooled, negative nasopharyngeal swab (NPS) matrix at designated concentrations. The results of the Stage 2 confirmation are seen in Table 1, indicating the established LoD for each qPCR instrument tested.

Table 1. ChromaCode HDPCR SARS-CoV-2 Assay – Direct protocol LoD

qPCR Instrument	LoD, Genomic Equivalents/mL
QuantStudio 7, 96 Well[1]	1000
QuantStudio 5, 384 Well[1]	1000
QuantStudio 5, 0.2mL Block[1]	1000
QuantStudio 12K Flex, 96 Well[1]	1000
7500 Fast Dx[1]	1000
7500 Fast[1]	3000

STUDY EVALUATION COMPARING RESPIRATORY SAMPLES

An evaluation of the Direct Extraction protocol on respiratory samples was conducted using the HDPCR SARS-CoV-2 Assay kit on three qPCR instruments. The samples 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.

Table 2. HDPCR SARS-CoV-2 Assay – Direct Protocol

qPCR Instrument	Comparator Result	Total	Correct	Incorrect	Percent Agreement	95% Confidence Interval
QuantStudio 5, 384-Well	Detected	30	28	2 (FN)	93.9%	76.5-98.8%
	Not Detected	30	29	1 (FP)	96.7%	81.0-99.8%
7500 Fast Dx	Detected	30	27	3 (FN)	90.0%	72.3-976.4%
	Not Detected	30	30	0 (FP)	100.0%	85.9-100.0%
7500 Fast	Detected	30	29	1 (FN)	96.7%	81.0-99.8%
	Not Detected	30	30	0 (FP)	100.0%	85.9-100.0%

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

INTERFERING SUBSTANCES

The Direct Extraction protocol was challenged by the presence of 13 potential interferents that can be present in respiratory samples to determine the impact to assay performance. All samples were tested at 3X LOD with 3 extraction replicates each having 2 PCR replicates (6 total replicates). The results are shown in Table 3 and demonstrate robust performance of the Direct Extraction protocol.

Table 3. Interfering Substances study results

Interferent	Concentration Tested	N1	N2	RNase P
Control (No Interferent)	N/A	12/12	12/12	12/12
Blood	2% (v/v)	6/6	6/6	6/6
Nasal Corticosteroid	5% (v/v)	6/6	6/6	6/6
Decongestant Nasal Spray	5% (v/v)	6/6	6/6	6/6
Human Genomic DNA	41.2 ng/rxn	6/6	6/6	6/6
Throat Lozenge	167 mg/mL	6/6	6/6	6/6
Mucin	60 µg/mL	6/6	6/6	6/6
Mupirocin	3.3 mg/mL	6/6	6/6	6/6
Oseltamivir	2.2 µg/mL	6/6	6/6	6/6
Phenylephrine	5% (v/v)	6/6	6/6	6/6
Saline	15% (v/v)	6/6	6/6	6/6
Tobramycin	4.0 µg/mL	6/6	6/6	6/6
Zanamivir	0.282 µg/mL	6/6	6/6	6/6
Zicam	5% (v/v)	6/6	6/6	6/6

CONCLUSION

The Direct Extraction protocol of ChromaCode's SARS-CoV-2 Assay is a sensitive test that eliminates the need for sample extraction and performs well against traditional extraction-based protocols. The inclusion of this protocol can reduce costs, reliance on extraction systems, and time associated with extraction-based protocols.

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