HDPCR™SARS-CoV-2 Assay

Instructions for Use – Version 14, February 2022

IVD

COVID-19 Emergency Use Authorization Only

For *in vitro* diagnostic (IVD) Use | Rx Only

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HDPCR™SARS-CoV-2 Assay

The HDPCR™ SARS-CoV-2 Assay is an *In vitro* diagnostic Real-Time PCR test for Coronavirus COVID-19.

Table 1. HDPCR SARS-CoV-2 Assay Information

COVID-19 Emergency Use Authorization Only. For *in vitro* diagnostic (IVD) Use | Rx Only		
Sample Types[1]	**Extraction Platform**	**qRT-PCR Instruments**
Nasopharyngeal swabs oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, and nasal wash	Thermo Scientific™ KingFisher™ Flex	Applied Biosystems™7500 Fast Applied Biosystems™7500 Fast Dx Applied Biosystems™QuantStudio™5 (96-Well, 0.2 mL Block and 384-Well Block) Applied Biosystems™QuantStudio™7 (96-Well Fast Block and 384-Well Block) Applied Biosystems™QuantStudio™12K Flex (96-Well Fast Block and 384-Well Block)

[1] The performance was established using nasopharyngeal swab specimen type collected in UTM or VTM. Anterior nasal swabs, oropharyngeal swabs, mid-turbinate nasal swabs, nasal aspirate and nasal wash are also considered acceptable specimen types for use with the HDPCR SARS-CoV-2 Assay, but performance has not been established.

HDPCR™SARS-CoV-2 Assay

For COVID-19 Emergency Use Authorization Only

Instructions for Use

Intended Use

The HDPCR™SARS-CoV-2 Assay is a reverse transcription real-time polymerase chain reaction (qRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs as well as nasal aspirate and nasal wash from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The HDPCR SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The HDPCR SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principles of Procedure

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 nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, and nasal wash using 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, effective 3/15/2020. 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. The N1 target is in the FAM channel, the N2 target is in the VIC channel, and the RNase P internal control (RNase P (IC)) is in the Cy5 channel.

Assay Layout and Controls

The HDPCR primer and probe formulation contains TaqMan probes at a reaction limiting concentration which, in combination with ChromaCode Calibrators, allows for the use of end-point fluorescence detection of targets. Five calibrators are run per qRT-PCR plate, including two no template, and three triple-target replicate calibrators. The median calibrator endpoint from all three positive calibrator wells is taken as the target endpoint for each of the color channels and used to scale and compare sample data. This also allows for

unification of expected values across instruments, just as a Ct does in traditional real-time PCR. Interpretation of these results is described below:

The ChromaCode Cloud software independently assesses if the SARS-CoV-2 targets amplified in a sample and if the RNase P (IC) passed or failed. To be detected, a target amplification must reach a scaled endpoint fluorescence that is closer to the positive calibrator for each channel than the negative calibrator for that channel– FAM channel for N1 and VIC channel for N2. If this value is not reached, the target is not represented as detected and listed as "not detected" in the generated report. The purpose of the RNase P (IC) is to confirm a negative sample result. If the endpoint value of the RNase P (IC) amplification is closer to positive calibrator in the Cy5 channel, the internal control passes. If its endpoint is closer to the negative calibrator in the Cy5 channel, and neither N1 or N2 are detected, the internal control fails, and the sample well is invalid. If its endpoint is not closer to the positive calibrator and either N1, N2, or both are detected, the internal control need not be assessed.

ChromaCode Cloud assesses run success of the positive run control, the negative run control, and the SARS-CoV-2 Assay Calibrators. The positive run control, COV_Pos, contains an RNA transcript of the Nucleocapsid gene and is in a matrix with human DNA. This control is added directly to the master mix on the PCR plate. One COV_Pos is run per qRT-PCR run to verify the master mix was appropriately made, by confirming reverse transcriptase activity and the PCR amplification of N1, N2 and RNase P (IC). All targets should amplify in the COV_Pos well. If this control fails, any or all of the three targets did not amplify, the plate is invalid. The negative run control (NTC) is added directly to the master mix on the PCR plate. One NTC is run per qRT-PCR run to confirm that there is no contamination in the master mix or plate set up. No targets should amplify in the NTC well. If this control fails due to aberrant amplification, the plate is invalid. A plate is marked as invalid if the SARS-CoV-2 Assay calibrators fail the established QC criteria.

The negative extraction control, COV_Neg, is human DNA in a stabilizing matrix. This control is processed like a specimen, as it goes through extraction and qRT-PCR to monitor for cross contamination and the successful extraction of nucleic acid. One COV_Neg is run in every unique extraction process represented on a SARS-CoV-2 run. This control must be manually interpreted. A successful COV_Neg would have amplification of only the RNase P (IC) target and no others. If the COV_Neg fails, the samples processed in the same extraction run also should be manually interpreted as failed.

An interpretation guide to the SARS-CoV-2 Assay Controls is found in Table 2.

Table 2. HDPCR SARS-CoV-2 Assay Controls

Control:	Controls for:	Control Requirement:
NTC (No Template Control)	Contamination in master mix/ plate set up	One per qRT-PCR Plate; Control Passed
COV_Pos (Positive Run Control)	qRT-PCR Process Control	One per qRT-PCR Plate; Control Passed
COV_Neg (Negative Extraction Control)	Extraction Control, qRT-PCR Process for RNase P	One Per Extraction; Control Passed
RNase P (in HDPCR Mix) in Negative Sample	Confirms full process for negative samples	Built in for all qRT-PCR Wells; Pass When Sample Negative

Materials Provided and Storage

The product is available in a low volume test configuration (PN: 0683) or a High Throughput (HT) test configuration (PN: 0904 and PN: 0905). To run the HDPCR SARS-CoV-2 Assay in an HT configuration, both PNs 0904 and 0905 are required. Control kits (PN: 0690) are available to be ordered and are required for both configurations.

Table 3. HDPCR SARS-CoV-2 Assay, 480 Tests (PN: 0683)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
HDPCR SARS-CoV-2 Subkit (480 Tests):	0682	1 Kit	N/A	2-8°C	-25 to -15°C
Enzyme Mix 03E	0688	5 Tubes	1050	2-8°C	-25 to -15°C
HDPCR SARS-CoV-2 Mix	0674	5 Tubes	450	2-8°C	-25 to -15°C
Reverse Transcriptase 01	0081	5 Tubes	120	2-8°C	-25 to -15°C
COV_A	0675	2 Tubes	80	2-8°C	-25 to -15°C
COV_B	0676	2 Tubes	80	2-8°C	-25 to -15°C
COV_C	0677	2 Tubes	80	2-8°C	-25 to -15°C
COV_D	0678	2 Tubes	80	2-8°C	-25 to -15°C
COV_E	0679	2 Tubes	80	2-8°C	-25 to -15°C

Table 4. HDPCR SARS-CoV-2 Box 1, HT Assay, 2592 Rxn (PN: 0904)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
Enzyme Mix 03E	0688	27 Tubes	1050	2-8°C	-25 to -15°C
HDPCR SARS-CoV-2 Mix	0674	27 Tubes	450	2-8°C	-25 to -15°C
Reverse Transcriptase 01	0081	27 Tubes	120	2-8°C	-25 to -15°C

Table 5. HDPCR SARS-CoV-2 Box 2, HT Calibrators, 36 Runs (PN: 0905)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
COV_A	0675	9 Tubes	80	2-8°C	-25 to -15°C
COV_B	0676	9 Tubes	80	2-8°C	-25 to -15°C
COV_C	0677	9 Tubes	80	2-8°C	-25 to -15°C
COV_D	0678	9 Tubes	80	2-8°C	-25 to -15 °C
COV_E	0679	9 Tubes	80	2-8°C	-25 to -15 °C

Table 6. HDPCR SARS-CoV-2 Controls, 20 Extraction Runs (PN: 0690)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
COV_Neg	0681	20 Tubes	200	Dry Ice	-25 to -15°C
HDPCR SARS-CoV-2 Pos Ctrl/ Diluent, 4ea:	0696	1 Kit	N/A	Dry Ice	-25 to -15°C
COV_Pos	0680	4 Tubes	70	Dry Ice	-25 to -15°C
Diluent	0695	4 Tubes	200	Dry Ice	-25 to -15°C

HDPCR SARS-CoV-2 Assay and Controls Kits should be stored between -25 and -15°C for up to 12 months from date of manufacture. Assay and Control Kits should not be used beyond expiration date listed on labels.

The HDPCR SARS-CoV-2 Mix, Enzyme Mix 03E and Reverse Transcriptase 01 may be thawed and used up to four (4) times, storing at -25 to -15°C between uses.

The COV_A, COV_B, COV_C, COV_D, and COV_E calibrators may be thawed and used up to 4 times, storing at -25 to -15°C between uses.

The COV_Pos may be thawed and used up to 4 times, storing at -25 to -15°C between uses.

The Diluent may be thawed and used up to 4 times, storing at -25 to -15°C between uses.

The COV_Neg is single use only and may not be refrozen.

Materials Required but Not Provided

Equipment:

- One qRT-PCR Instrument
- Extraction System

Table 7. Supported Extraction System

Instrument	Catalog No.
Thermo Scientific™ KingFisher™ Flex Extraction System	5400630

Table 8. Supported qRT-PCR Instruments

Instrument	Software	Catalog No.
Applied Biosystems™ 7500 Fast[2]	2.3	4351106
Applied Biosystems™ 7500 Fast Dx[3]	1.4.1	4406984
Applied Biosystems™ QuantStudio™ 5, 96-Well, 0.2 mL[2]	1.4.3	A28569
Applied Biosystems™ QuantStudio™ 5 Flex, 384-Well[2]	1.5.1	A28140
Applied Biosystems™ QuantStudio™ 7 Flex, 96-Well[2]	1.3	4485698
Applied Biosystems™ QuantStudio™ 7 Flex, 384-Well[2]	1.3	4485695
Applied Biosystems™ QuantStudio™ 12K Flex, 96-Well[2]	1.3	4471088
Applied Biosystems™ QuantStudio™ 12K Flex, 384-Well[2]	1.2.2	4471134

- Vortex mixer
- Mini centrifuge
- PCR plate centrifuge
- Pipettes for volumes 5 to 1000 µL

Consumables:

- Molecular Grade RNase/ DNase Free Water (for No Template Control)
- DNase/ RNase free, sterile, filter tips for volumes 5 to 1000 µL
- DNase/ RNase free, sterile tubes
    - 2 mL tubes
- Disposable gloves

[2] This instrument requires qualification prior to use with the HDPCR SARS-CoV-2 EUA Assay. Please refer to "Appendix 1: Applied Biosystems Real-Time PCR Systems Qualification" section of this document for the required protocol and acceptance criteria. The appropriate label to affix to these instruments upon qualification is found in "Appendix 2: RUO Applied Biosystems Real-Time PCR Systems Qualification Additional Label for RUO Applied Biosystems Real-Time PCR Systems" section of this document.
[3] If using the Applied Biosystems™ 7500 Fast Dx, the user must also download 7500 Software v2.3 for run file conversion.

Table 9. Specific Consumables

Consumables	Catalog No.
Applied Biosystems™ MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plate with Barcode	4483485
Applied Biosystems MicroAmp Optical 96-Well Reaction Plate (for Applied Biosystems™ QuantStudio™ 5 Flex Software Version 1.4.3; 96-Well, 0.2 mL: Catalog Number A28569)	N8010560
Applied Biosystems MicroAmp Optical 384-Well Reaction Plate with Barcode	4326270
Bio-Rad Hard-Shell® 384-Well PCR Plates (for Applied Biosystems™ QuantStudio™ 5 Flex Software Version 1.5.1; 384-Well: Catalog Number A28140)	HSP3805
Applied Biosystems™ MicroAmp™ Optical Adhesive Film PCR/Real-time PCR Compatible	4311971
Thermo Scientific™ KingFisher™ Flex Extraction System	
Applied Biosystems™ MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	A42352
KingFisher 96 well accessory kit from Macherey Nagel	744951

Warnings and Precautions

There are no known hazardous substances included in the manufacture of the HDPCR SARS-CoV-2 Assay. Safety Data Sheets are available online at https://chromacodecloud.com/downloads or through ChromaCode Customer Support at customer.support@chromacode.com

Additional material or chemicals required for the use of the HDPCR SARS-CoV-2 Assay should be closely examined by the user. The user should carefully read all warnings, instructions or Safety Data Sheets provided by the supplier and follow the general safety precautions when handling biohazards, chemicals, and other materials.

General Precautions

- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3 (b)(1), unless the declaration is terminated, or authorization is revoked sooner.
- Standard precautions and procedures should be taken when handling and extracting human samples.
- Standard precautions and procedures should be taken when using extraction instruments.
- Standard precautions and procedures should be taken when disposing of samples, extracted material and waste.
- Dispose of reagents according to local regulations.

- Do not use reagents after their recommended stability time frame.
- Do not mix reagent lots from different HDPCR SARS-CoV-2 Assay kits.
- Avoid contamination by following good laboratory practices, wearing proper personal protective equipment, segregating workflow, and decontaminating workspace appropriately.
- Ensure all consumables are DNase and RNase free.

Specimen Collection and Storage

Upper respiratory samples should be collected using standard procedures and recommendations from the collection device manufacturer. Swab specimens can be collected in UTM or VTM. Specimens should not be collected in saline.

Please refer to the Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation for the 2019 Novel Coronavirus (2019-nCoV) provided by the CDC, www.cdc.gov.

- Samples can be stored at 2-8°C for 72 hours after collection prior to extraction. If samples need to be transported, maintain 2-8°C on ice packs for overnight shipment.
- For longer term storage, unextracted samples can be stored at ≤-70°C. If samples need to be transported, maintain ≤-70°C on dry ice for overnight shipment.
- Extracted nucleic acids can be stored at ≤-70°C. If samples need to be transported, maintain ≤-70°C for overnight shipment.

Note: Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.

Running a New Test

Preparing to Run Assay on Instrument for the First Time

Prior to starting runs on any new instrument, the instrument must be registered on ChromaCode Cloud using the instrument's serial number and the appropriate run template file for that instrument must be downloaded. Prior to starting runs on any new Research Use Only instrument, the instrument must be qualified under the direction in Appendix 1 and a label must be affixed to the side of the instrument per Appendix 2.

Note: Any instrument running the HDPCR SARS-CoV-2 Assay must be calibrated for the following dyes: FAM, VIC, and Cy5

Registering an Instrument on ChromaCode Cloud

Once your institution has been given access to ChromaCode Cloud, the administrator will be able to upload lab instrument(s) to analyze HDPCR data.

1. Open a new window in Google Chrome™, or other ChromaCode Cloud compatible browser, and navigate to https://chromacodecloud.com. If you wish to bookmark the web application, log in to ChromaCode Cloud and bookmark the landing page that displays the *browse for instrument file to import* plate.
2. Click the **Admin** link at the top of the page.
3. Select **Add Instrument** at the top right of the page.

   Note: The user will need to have been designated as the administrator to be able to access this functionality.
4. Select the instrument model type in the drop-down menu labeled **Model**.
5. Enter a desired lab nickname for the instrument.
6. Enter the instrument's serial number; the number can be found on the side of the instrument.
7. Select **Save Instrument**.
8. Your instrument should now be listed on the page with a green checkmark indicating *equalization not required*.

Download the Template Run File

The Template File contains all the parameters preconfigured to run the HDPCR SARS-CoV-2 Assay, including the run parameters:

Table 10. Thermal Cycling Conditions for HDPCR SARS-CoV-2 Assay

Stage	Temperature(°C):	Time:	Reps:
1	50.0	15:00	1
2	95.0	2:00	1
3	95.0	0:03	55
	58.0*	1:00	

*This step should be the optical read step

To download the Template Run File:

1. On ChromaCode Cloud, navigate to **Downloads**.
2. Download the appropriate .edt or .sdt template run file for your instrument type and save the template file on your instrument.
   Note: Users need only download the template file and save to their instrument upon first use.

Table 11. Template Files for HDPCR SARS-CoV-2 Assay

Instrument	.edt/.sdt filename
Applied Biosystems™ 7500 Fast	COVEUA12_7500Fast_template
Applied Biosystems™ 7500 Fast Dx	COVEUA12_7500FastDx_template
Applied Biosystems™ QuantStudio™ 5 96-Well, 0.2mL	COVEUA12_QS5_96well_template
Applied Biosystems™ QuantStudio™ 5 384-Well	COVEUA12_QS5_384well_template
Applied Biosystems™ QuantStudio™ 7 96-Well	COVEUA12_QS7_96well_template
Applied Biosystems™ QuantStudio™ 7 384-Well	COVEUA12_QS7_384well_template
Applied Biosystems™ QuantStudio™ 12K Flex 96-Well	COVEUA12_QS12K_96well_template
Applied Biosystems™ QuantStudio™ 12K Flex 384-Well	COVEUA12_QS12K_384well_template

Applied Biosystems™ 7500 Fast Template File

1. Open the appropriate template, **COVEUA12_7500Fast_template** on your instrument.
2. Under the **Setup** tab on the left side, select **Experiment Properties**.
3. Add your instrument's serial number and assay version (COVEUA12) to the comments field.
   a. Replace **InstrumentSerialNumber** on a 7500 Fast Instrument with your instrument's serial number and assay version
   EX: 1234567890_COVEUA12

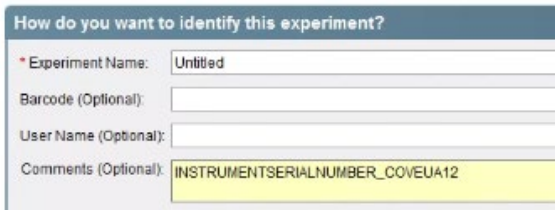


Figure 1. Update comments field to contain your instrument serial number and assay version

4. Select **File** on the Navigation tab and select **Save as Template**.
5. Name the file **COVEUA12_7500FAST_template.edt** and save in a preferred location.

Applied Biosystems™ 7500 Fast Dx, QuantStudio™ 12K Flex, QuantStudio™ 5 96-Well 0.2mL and 384-Well, and QuantStudio™ 7 96- and 384-Well Template Files

1. Open the COVEUA12 template file on your instrument and proceed to running HDPCR SARS-CoV-2 Assay.

Note: You must manually add the comments field each time when using the Applied Biosystems 7500 Fast Dx.

Sample Preparation

Refer to the Thermo Scientific™ KingFisher™ Flex User Manual for full system usage and maintenance details. Use the following protocol and isolation kit depending on PCR instrument selected:

- MVP_Flex Protocol with the Applied Biosystems MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Product Number A42352) on the Thermo Scientific KingFisher Flex System

Overview:

1. Vortex primary specimen container to homogenize.
2. Add 200 µL of the specimen to extraction cartridge.
3. Extract in accordance with standard procedure, eluting into 50 µL.
4. Include one COV_Neg control on every extraction run, treating the control the same way as a specimen.

   Note: Store COV_Neg at 4°C once thawed and use within 1 day of thawing. This control is single use only and may not be refrozen.
5. Store the extracted samples on cold blocks or ice if will be used immediately, otherwise freeze at ≤-70°C.

Create the Plate Layout Map

1. Open provided template file on thermal cycling instrument.
2. Assign a well for each of the samples.
3. Assign a well for each of the 5 HDPCR SARS-CoV-2 calibrators.

COV_A COV_D
COV_B COV_E
COV_C

4. Assign a well for all controls: COV_Pos for the positive run control, COV_Neg for the negative extraction control(s), and NTC for the no template control.
   Note: There must be one positive run control per qRT-PCR plate, one negative extraction control per extraction run, and one NTC per qRT-PCR plate.
   Note: If COV_Pos and NTC are not named exactly as such, the software will not recognize them as controls and will not interpret them accordingly.
5. For the COV_Neg control, you may append the COV_Neg name to associate with a specific extraction run, as samples from more than one extraction can be run on the same plate.
6. Use your plate layout to load your samples, calibrators, and controls after preparing the amplification reaction mix.

Prepare the Amplification Reaction Mix

Note: Prepare the amplification reaction mix in a pre-PCR area.

Thaw the following components, as described in Table 12, at room temperature until no ice crystals remain:

Table 12. Amplification Reaction Mix Components

Component
Enzyme Mix 03E
HDPCR SARS-CoV-2 Mix
Reverse Transcriptase 01

1. Vortex the HDPCR SARS-CoV-2 Mix for 5 seconds and spin to remove liquid from the cap.
2. Gently invert the Reverse Transcriptase 01 and Enzyme Mix 03E 5 times and spin to remove liquid from the cap.
3. Prepare the amplification reaction mix in a 2 mL tube according to Table 13, where n = the number of reaction wells to be run.

   Note: Remember to include all calibrators and controls in the calculation

Table 13. Amplification Reaction Mix Component Calculations

Component	Volume
Enzyme Mix 03E	(n+5) x 10 µL
HDPCR SARS-CoV-2 Mix	(n+5) x 4 µL
Reverse Transcriptase 01	(n+5) x 1 µL

4. Vortex amplification reaction mix for 5 seconds and spin down to remove liquid from the cap.
5. Aliquot 15 µL of the amplification mix into each well that will be used for the run; use caution while loading to avoid introduction of bubbles into the well.

Prepare COV_Pos Positive Control

Note: Prepare COV_Pos Positive Control in a template positive area. The dilution of COV_Pos must be made fresh for each run of the HDPCR SARS-CoV-2 Assay and used within 1 hour of dilution. The dilution must be discarded after use.

1. Thaw the COV_Pos control and the Diluent.
   Note: Return the individual COV_Pos and Diluent tube to -20°C if tubes will be used again. COV_Pos and Diluent may only be thawed and used up to 4 times.
2. Vortex for 5 seconds and spin down to remove liquid from the cap.
3. Retrieve fresh, DNase/RNase free, sterile tube and add 45 µL of Diluent
4. Add 5 µL of COV_Pos to the Diluent, creating a positive control of approximately a 100 copy/reaction.
5. Vortex for 5 seconds and spin down to remove liquid from the cap.

Add Samples and Calibrators to Plate

Note: Prepare and add samples and calibrators to plate in template positive area and keep samples on cold block or ice throughout plate set up

1. Thaw the calibrators and the extracted samples (including extracted COV_Neg) if previously frozen.
2. Vortex for 5 seconds and spin down to remove liquid from the cap.
3. Add 5 µL of each calibrator to the well in accordance to the plate layout map.
   Note: All 5 calibrators must be run on every plate
4. Add 5 µL of the diluted COV_Pos and extracted COV_Neg controls to the wells in accordance with the plate layout map.
5. Add 5 µL of each sample to the wells in accordance with the plate layout map.
6. Add 5 µL of molecular grade water as the NTC in accordance with the plate layout map.
7. Place the film on top of the plate and use the squeegee to adhere the film, especially around the edges to avoid evaporation.
8. Spin the plate for 1 minute in a PCR plate spinner.

Create a Run File and Start the Run

Refer to Instrument User Manuals for full system usage and maintenance details.

1. On the instrument software, open the provided instrument specific SARS-CoV-2 template, as referenced in Table 12.

Note: At the start of each run, default comments on a 7500 Fast Dx Instrument must be replaced to have InstrumentSerialNumber_COVEUA12 (EX: 1234567890_COVEUA12)

2. Ensure the Sample, Calibrator, and Control Names are correctly entered in software based on the Plate Layout Map.
3. Start the run in the software.

ChromaCode Cloud Data Analysis

ChromaCode Cloud supports .eds and .xls file types from the supported instruments and software versions listed in the Materials Required section of this document. Customers must confirm, as part of their laboratory assay validation process, that the files generated from their instruments are compatible for upload to ChromaCode Cloud. Contact Technical Support with any questions on confirming ChromaCode Cloud file compatibility. The following provides specific instructions for saving and/or exporting data.

ABI 7500 Fast, QuantStudio 5, QuantStudio 7 and QuantStudio 12k Flex

Ensure that .eds run files from instruments with a touch screen capability are saved directly from the computer attached to the instrument rather than the touchscreen monitor on the instrument.

The .eds file saved from the instrument computer can be uploaded for analysis.

Alternatively, an .xls file may be exported for analysis as follows:

1. Upon run completion, export the .xls run file by navigating to the **Export** tab, selecting .xls as **File Type**.
2. Select **Sample Setup, Raw Data**, **Amplification**, **Multicomponent**, and **Results.**
3. Select a **location** for the file to be saved and then select **Start Export.**
4. Upload the .xls file to ChromaCode Cloud for analysis.

ABI 7500 Fast Dx

1. Once your run has completed on the 7500 Fast Dx instrument, save your .sds run file to a known location.
2. If necessary, transfer your .sds file to a computer that has the 7500 Software v2.3 installed.
3. Open the .sds file in the 7500 Software v2.3.
4. Confirm that the comments field contains the correct string (InstrumentSerialNumber_COVEUA12).
5. Navigate to **File, Save As**, and save the file with a .eds extension to a known location.

Results Interpretation

SARS-CoV-2 N1, N2, and RNase P (IC) calls are determined by the ChromaCode Cloud software.

Upload the Run Data to ChromaCode Cloud

1. Open a new window in Google Chrome, or another ChromaCode Cloud compatible browser, and navigate to https://chromacodecloud.com
2. Log into ChromaCode Cloud.
3. Once on the home page, select **browse for instrument file to import**.

Figure 2. Browse for Instrument file to Import

4. Browse to find the desired .eds run file to upload for analysis.
5. Select **Open** to begin analysis.

Note: ChromaCode Cloud performs file integrity checks as part of the upload process. However, to reduce potential errors, upload file directly from instrument to ChromaCode Cloud. Do not open file on any additional software between completion of run and uploading to ChromaCode Cloud.

Plate Quality Control

Note: The plate QC status and controls status must be assessed before sample result interpretation.

ChromaCode Cloud automatically assesses the plate QC status. This information is provided in a Plate Summary view and a Well Details page on ChromaCode Cloud. Additionally, this information is consolidated in an exportable portable document format (pdf) report. The report can be downloaded from the Plate Summary page by clicking **view report** as seen in Figure 3.

Figure 3. Plate Passing ChromaCode Cloud QC

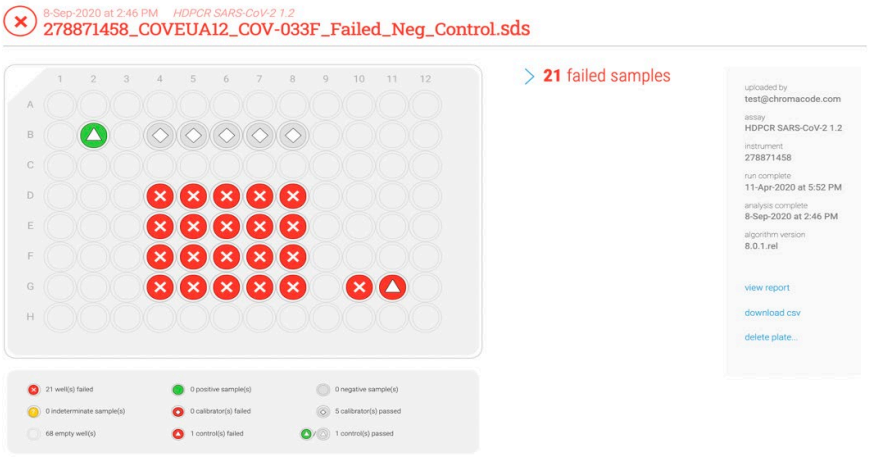


Figure 4. Plate Summary Page with Failing Plate QC

HDPCR SARS-CoV-2 calibrators must be run and pass quality control checks on every plate run. If these calibrators do not pass quality control checks, the plate run will be automatically flagged as failed on ChromaCode Cloud by a red X next to the run name, which will be written in red text (an example of a failed plate in Figure 4). A plate that has passed plate QC will have a green check and the run name will be written in green text in Figure 3.

COV_Pos will be interpreted by the ChromaCode Cloud software, if named COV_Pos. If this control passes, it will be represented as a green well with a triangle shape. If this control fails, it will be represented as a red well with a triangle shape and the plate will fail.

The No Template Control (NTC) will be interpreted by the ChromaCode Cloud software if named NTC. If this control passes, it will be represented as a grey well with a triangle shape. If this control fails, it will be represented as a red well with a triangle shape and the plate will fail.

Note: If the No Template Control (NTC) and Positive Run Control (COV_Pos) are named anything other than "NTC" and "COV_Pos", respectively, they will not be interpreted as part of the ChromaCode Cloud QC analysis.

The COV_Neg control must be interpreted manually by the end user. To pass, this controls requires amplification of the RNase P (IC) and no amplification of the SARS-CoV-2 targets. The control passes if ChromaCode Cloud determines this well to be negative for the SARS-CoV-2 targets (viewed on the plate summary page or by generating a PDF results report).

A summary of the expected results for each control is summarized in the table below, along with action to take if a control does not have the expected results.

Table 14. Plate QC Controls

Control	Passing Result	ChromaCode Cloud Text (Well Details Page)	Action if Fail
NTC (No Template Control)	No Target Detection	control passed	Plate Invalid: repeat run with fresh NTC
COV_Pos (Positive Run Control)	CoV-2 N1 detected CoV-2 N2 detected RNase P (IC) Passed	control passed	Plate Invalid: repeat run with fresh COV_Pos.
COV_Neg (Negative Extraction Control)	CoV-2 N1 not detected CoV-2 N2 not detected RNase P (IC) Passed	no targets detected Internal Control Passed	Sample Failure: for the samples run in the same extraction as the control. Repeat extraction for these samples and rerun.

Sample Results Interpretation

ChromaCode Cloud automatically assesses the amplification and detection of the N1 and N2 targets and the RNase P (IC) internal control status for each sample. This information is provided in a Plate Summary view (Figure 3) and a Well Details page. Additionally, this information is consolidated in an exportable report.

The end user must interpret the SARS-CoV-2 status of each sample based on N1, N2 and the RNase P (IC) status as presented in Table 15.

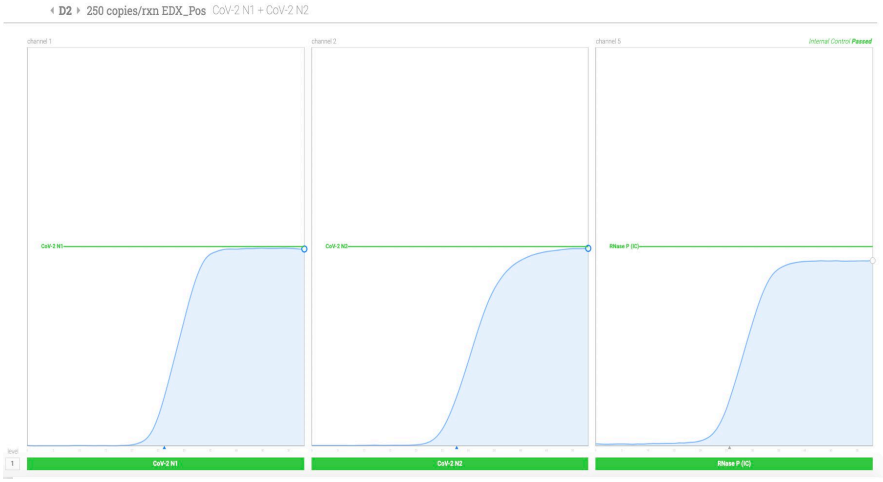


Figure 5. Well Details Page Containing Targets

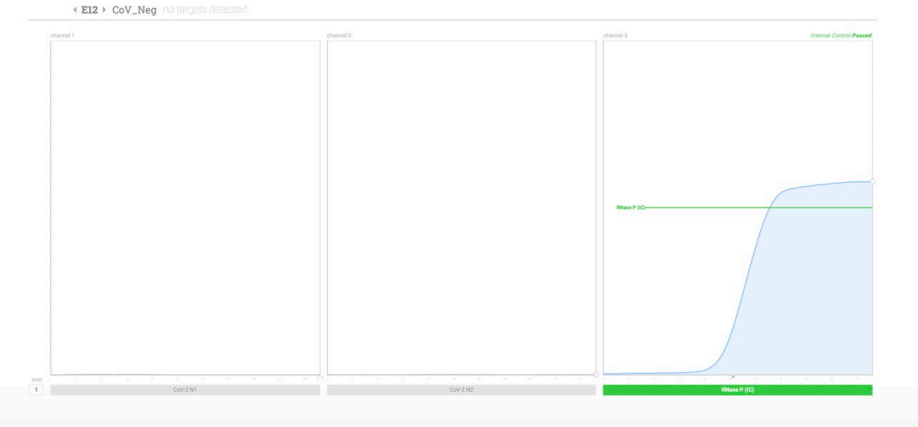


Figure 6. Well Details Page Containing Negative Sample

The Well Details page of any individual sample will highlight the target name listed under the channel in which that target is found in green if that target is detected. Additionally, detected targets (CoV-2 N1 and CoV-2 N2) are listed next to the sample name above the amplification curves (Figure 5). If there is no amplification, the target name listed under the channel will remain gray and there will be either only one target listed next to the sample name, or there will be "no targets detected" listed next to the sample name (Figure *6*). There is text above the amplification curve of Channel 5 which indicates whether the Internal Control has succeeded by describing it as either "Internal Control Passed," "Internal Control Failed," or "Internal Control Not Assessed."

Reports can be generated through ChromaCode Cloud.

The Run Summary report shows

- SARS-CoV-1 N1 or SARS-CoV-N2 when a target is detected
- NO TARGETS DETECTED when no targets are detected

The Sample Details Report is generated for every well and shows

- SARS-CoV-2 N1 as DETECTED or NOT DETECTED
- SARS-CoV-2 N2 as DETECTED or NOT DETECTED
- Human RNase P (IC) as PASSED, FAILED, or NOT ASSESSED

Table 15 outlines the expected results from the assay and potential recourse that must be taken.

Table 15. HDPCR SARS-CoV-2 Results Interpretation

ChromaCode Analysis Output (Sample Detail Report)			Laboratory Interpretation & Actions	
SARS-CoV-2 N1	SARS-CoV-2 N2	Human RNase P (IC)	Report	Action
Detected	Detected	Passed or Not Assessed	SARS-CoV-2 Positive	Report result to appropriate health authorities.
Detected	Not Detected	Passed or Not Assessed	SARS-CoV-2 Presumptive Positive	Repeat testing of nucleic acid and/ or re-extract and repeat HDPCR SARS-CoV-2. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
Not Detected	Detected	Passed or Not Assessed	SARS-CoV-2 Presumptive Positive	Repeat testing of nucleic acid and/ or re-extract and repeat HDPCR SARS-CoV-2. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
Not Detected	Not Detected	Passed	SARS-CoV-2 Negative	Report result to appropriate health authorities.
Not Detected	Not Detected	Failed	Invalid Results	Repeat test, if second test result is invalid, report as invalid and recommend recollection if patient is still clinically indicated.

Limitations

The use of this assay as an *in vitro* diagnostic under the FDA COVID-19 Emergency Use Authorization (EUA) is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity test by Rx only.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may lead to erroneous results.

The performance of HDPCR SARS-CoV-2 Assay was established using nasopharyngeal swab specimen type collected in UTM or VTM. Anterior nasal swabs, oropharyngeal swabs, mid-turbinate nasal swabs, nasal aspirate, and nasal wash specimens are also considered acceptable specimen types for use with the HDPCR SARS-CoV-2 Assay, but performance has not been established for these specimen types.

Samples must be collected according to manufacturer recommended protocols and transported and stored as described herein.

Samples should not be collected in saline.

The HDPCR SARS-CoV-2 Assay performance was established using the Thermo Scientific KingFisher Flex and the Applied Biosystems MagMAX Viral/ Pathogen Nucleic Acid Isolation Kit with the MVP_Flex protocol. Other extraction instrumentation and kits have not been tested with this assay.

All instrumentation used with the HDPCR SARS-CoV-2 Assay kits must be up to date on normal preventative maintenance and servicing/ calibration schedules, including calibration with the FAM, VIC and Cy5 fluorescent dyes on the ABI 7500 Fast, ABI 7500 Fast Dx, QuantStudio 5, QuantStudio 7 Flex and QuantStudio 12K Flex real time PCR systems.

The effects of interfering substances have not been assessed with the HDPCR SARS-CoV-2 Assay.

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs on the HDPCR SARS-CoV-2 Assay have not been evaluated.

False-positive results may arise from various reasons, including, but not limited to the following:

- Contamination during specimen collection, handling, or preparation
- Contamination during assay preparation
- Incorrect sample labeling

False-negative results may arise from various reasons, including, but not limited to the following:

- Improper sample collection or storage
- Degradation of SARS-CoV-2 RNA
- Presence of inhibitory substances
- Use of extraction reagents or instrumentation not approved with this assay
- Incorrect sampling window
- Failure to follow instructions for use
- Mutations In SARS-CoV-2 target sequences

Nucleic acid may persist even after the virus is no longer viable.

All results must be reported to appropriate public health authorities, following state and national guidelines.

Negative test results do not exclude possibility of exposure to or infection with SARS-CoV-2 virus. Patient handling will be directed by healthcare professionals.

Conditions of Authorization for the Laboratory

The HDPCR SARS-CoV-2 Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:
https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas
To assist clinical laboratories running the HDPCR SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed verbatim below and are required to be met by laboratories performing the EUA test:

A. Authorized laboratories[4] using your product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and you (local technical support center via email: technical.support@chromacode.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

[4] Your product refers to the HDPCR SARS-CoV-2 Assay. The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

G. ChromaCode, Inc, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

Performance Evaluation

The following data demonstrate the performance of the HDPCR SARS-CoV-2 Assay. All sample extractions used 200 µL of specimen input eluting into 50 µL using the KingFisher MVP_Flex Protocol with the Fisher MagMAX Viral/ Pathogen Nucleic Acid Isolation Kit.

Analytical Sensitivity: Limit of Detection (LoD)

The LoD of the HDPCR SARS-CoV-2 Assay was determined for the eight instruments in Table 16 for both N1 and N2 targets. The LoD was established by testing 20 replicates in 3X dilutions. The testing used heat-inactivated SARS-CoV-2 virus spiked into negative clinical nasopharyngeal swab specimen matrix (in UTM) at the designated concentrations. The LoD was defined as the concentration with greater than or equal to 95% positive reactions with a minimum of 20 valid wells. The lowest concentrations to detect 19/20 replicates or higher are shown in Table 16, indicating the established LoD for each qPCR instrument tested.

Table 16: Limit of Detection Confirmation Testing Summary

Instrument	LoD (GE/mL)	Detection Rate: CoV-2 N1	Detection Rate: CoV-2 N2
ABI 7500 Fast	333	20/20	19/20
ABI 7500 Fast Dx	900	20/20	20/20
ABI QuantStudio 5 (96-Well 0.2 mL Block)	333	19/20	20/20
ABI QuantStudio 5 (384-Well Block)	1000	20/20	19/20
ABI QuantStudio 7 (96-Well Block)	333	20/20	20/20
ABI QuantStudio 7 (384-Well Block)	2700	20/20	19/20
ABI QuantStudio 12K (96-Well Block)	333	20/20	20/20
ABI QuantStudio 12K (384-Well Block)	1000	20/20	20/20

Inclusivity: Analytical Sensitivity

An *in silico* inclusivity analysis was performed 12 November, 2021 using high-quality sequences in the GISAID database. A high-quality genome is defined by GISAID as <1% Ns and <0.05% unique non-synonymous mutations. Greater than 95% of sequences from GISAID have 100% homology to N1 and N2 oligonucleotides based on a bioinformatics assessment performed on 12 November 2021. Greater than 99.0% of sequences

from GISAID as of 12 November 2021 are predicted to amplify and be detected by the HDPCR SARS-CoV-2 Assay with N1 and N2 targets.

- The sequence alignment showed that the forward primer for the N1 target had a complete match with 97.03% of all sequences and a single mismatch with 2.96% of all sequences. The probe for the N1 target had a complete match with 98.60% of all sequences, a single mismatch with 1.37% of all sequences, two mismatches with 0.02% of all sequences, and three or more mismatches with 5.56E-5% (87/1,563,718) of all sequences. The reverse primer for the N1 target had a complete match with 99.57% of all sequences, a single mismatch with 0.43% of all sequences, two mismatches with 1.47E-5% (23/1,563,718) of all sequences, and three or more mismatches with 1.41E-5% (22/1,563,718) of all sequences.
- The sequence alignment showed that the forward primer for the N2 target had a complete match with 99.48% of all sequences and a single mismatch with 0.52% of all sequences, two mismatches with 1.54E-5% (24/1,563,718), and three or more mismatches with 3.84E-6% (6/1,563,718) of all sequences. The probe for the N2 target had a complete match with 99.47% of all sequences, a single mismatch with 0.52% of all sequences, two mismatches with 0.01% of all sequences, and three or more mismatches with 4.35E-5% (68/1,563,718) of all sequences. The reverse primer for the N2 target had a complete match with 99.65% of all sequences, a single mismatch with 0.34% of all sequences, and two or more mismatches with 2.56E-5% (40/1,563,718) of all sequences.

The risk of a loss of reactivity based on a single mismatch in a primer is low. Sequences having 1 mismatch more than 5 bases from the 3' end in a primer are predicted to have insignificant impact on the assay performance. Additionally, this risk of loss of reactivity is reduced having 2 different targets in the assay. Sequences having 2 mismatches in a primer (not within the last 5 bases of the 3'end) or a single mismatch in a probe should still be inclusive, albeit potentially at a higher limit of detection. Sequences predicted to be detected by the HDPCR SARS-CoV-2 Assay have a maximum of two mismatches in a primer (not within the last 5 bases of the 3'end) and a maximum of a single mismatch in the probe. Sequences with more mismatches are very rare and may cause sporadic false negatives or presumptive positives on the HDPCR SARS-CoV-2 Assay. Additionally, the primers have been designed to have melting temperatures several degrees above the annealing temperature used in the assay allow for 1 to 2 mismatches to be better tolerated.

Cross-Reactivity: Analytical Specificity

BLASTn analysis queries of the 2019-nCoV qRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 10/03/2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

2019-nCoV_N1 Assay:
Probe sequence of 2019-nCoV qRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. When combining primers and probe, there are no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive qRT-PCR results.

2019-nCoV_N2 Assay:

The forward primer sequence of 2019-nCoV qRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. When combining primers and probe, there is no prediction of potential false positive qRT-PCR results.

In summary, the 2019-nCoV qRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive qRT-PCR results.

Clinical Performance Evaluation

Clinical Evaluation of the HDPCR SARS-CoV-2 Assay was conducted on the Applied Biosystems 7500 Fast Dx, the QuantStudio 7 96- and 384-Well formats, and the QuantStudio 12K 384-Well instruments. A cohort of 160 natural nasopharyngeal swab specimens (80 negative and 80 positive) collected in universal transport media were enrolled using an FDA-authorized real-time RT-PCR assay. Specimens to be run on the HDPCR SARS-CoV-2 Assay were extracted using the KingFisher MVP_Flex Protocol with the Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit. The results of these clinical specimens are seen in Table 17.

Table 17: Clinical Performance Evaluation

Instrument	Comparator Result	Total	Correct	Incorrect	Invalid	Percent Agreement (95% CI)
7500 Fast Dx	Positive	80	80	0	0	100%(94.29-100%)
	Negative	80	79	1 (FP)[1]	0	98.75%(92.27-99.93%)
QS7,96-well	Positive	80	80	0	0	100%(94.29-100%)
	Negative	80	79	1 (FP)[1]	0	98.75%(92.27-)99.93%
QS7,384-well	Positive	80	79	1 (FN)[2]	0	98.75%(92.27-99.93%)
	Negative	79*	77	2 (FP[1],PP[3])	1	97.47%(90.31-99.56%)
QS12,384-well	Positive	80	80	0	0	100%(94.29-100%)
	Negative	80	79	1 (FP)[1]	0	98.75%(92.27-99.93%)

*RCOV0030 invalid on initial run and repeat
1. RCOV0201 Positive on all 4 instruments
2. RCOV0061 Presumptive positive on initial run and negative on rerun
3. RCOV0192 Presumptive positive on initial run and presumptive positive on repeat

Document Revision History

Document ID 334738

1	21 April 2020	Original IFU pre-sub to FDA
2	29 April 2020	Updated to include the following instrumentation • Applied Biosystems QuantStudio 12K qPCR instrument • Thermo Fisher KingFisher extraction platform
3	10 June 2020	• Updated to include final Emergency Use Authorization
4	11 September 2020	• Inclusion of FDA SARS-CoV-2 Reference Panel Testing
5 - 13	-	• ChromaCode updates to the HPDCR SARS-CoV-2 Assay while the FDA conducted its review; modifications implemented per "Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)"
14	14 February 2022	• Expansion to include the following qPCR instruments: • Applied Biosystems QuantStudio 12K Flex 384-Well • Applied Biosystems QuantStudio 7 384-Well • Applied Biosystems QuantStudio 5 384-Well • Applied Biosystems QuantStudio 5 96-Well (0.2 mL) • Applied Biosystems 7500 Fast Dx • Applied Biosystems 7500 Fast Dx .sds file export workflow updated • Language added regarding data file validation for ChromaCode Cloud • Inclusion of saline limitation • Inclusion of High Throughput (HT) Kit configuration option • Addition of Research Use Only Instrument Qualification and Labeling Appendices • Updated Inclusivity: Analytical Sensitivity • Updates to Inclusivity • Updated to include viral mutation limitations per EUA Conditions of Authorization • Revisions to Cross-Reactivity: Analytical Specificity to include information from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR IFU • Removed Roche MagNA Pure Extraction References • Updated Analytical Sensitivity: Limit of Detection • Updated Clinical Performance Evaluation

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and the instrument used were the Roche MagNA Pure 24 Pathogen 200 2.0 Protocol with the Total Nucleic Acid Isolation Kit with qRT-PCR on the Applied Biosystems 7500 Fast instrument. The results are summarized in Table 14.

Table 14 Summary of LoD Confirmation Results using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by the FDA	Specimen Type	HDPCR SARS-CoV-2 Assay LoD	Cross-Reactivity
SARS-CoV-2	NPS in VTM	5.4×10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

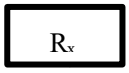
NDU/mL: RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Trademarks

HDPCR™ is a trademark of ChromaCode, Inc.
All other product names and trademarks are the property of their respective owners.

Explanation of Symbols

The following symbols are present on the HDPCR™ SARS-CoV-2 Assay labels.

Symbol	Definition
	Manufactured By
	Upper and Lower Storage Temperature Limitation
	By Prescription Only
	Lot Number
	Quantity

Symbol	Definition
	Product Catalog Number
	Use by Date
	In vitro Diagnostic Medical Device
	Information

Manufacturing and Distribution Information

Manufactured by
ChromaCode, Inc.
2330 Faraday Ave, Suite 100
Carlsbad, CA 92008 US

Support

E-mail: technical.support@chromacode.com
Phone: +1 442-244-4370

Sales and Marketing

E-mail: For orders please contact orders@chromacode.com
For general information please contact customer.support@chromacode.com
Phone: +1 442-244-4357

Appendix 1: RUO Applied Biosystems™ Real-Time PCR Systems Qualification

Prior to running the HDPCR SARS-CoV-2 EUA Assay for diagnostic testing on a Research Use Only (RUO) instrument, a qualification using the HDPCR SARS-CoV-2 Controls, 20 Extraction Runs (PN: 0690) must be performed as described in this section.

Required Materials

All required materials for sample preparation necessary for this qualification are listed in the following table. These materials from the HDPCR SARS-CoV-2 Controls Kit should be stored and handled at appropriate temperatures, as described in the Instructions for Use Materials Provided and Storage section. The extracted qualification samples should be kept cold during preparation and use.

Item	Part Number	QTY
COV_Neg	0681	5 Tubes
COV_Pos	0680	2 Tubes
Diluent	0695	5 Tubes

Instructions for Preparing Qualification Specimens Before Extraction

1. Thaw the COV_Neg, COV_Pos, and Diluent.
2. Vortex for 5 seconds and spin down to remove liquid from the cap.
3. Label 3 RNase/ DNase-free microfuge tubes as A, B, and C.
4. Aliquot 975 µL of Diluent into Tube A, then add 25 µL of COV_Pos.
5. Vortex Tube A for 5 seconds and spin down to remove liquid from the cap.
6. Aliquot 900 µL of Diluent into Tube B, then add 100 µL of Tube A.
7. Vortex Tube B for 5 seconds and spin down to remove liquid from the cap.
8. Aliquot 1 mL of COV_Neg into Tube C.
9. Vortex Tube C for 5 seconds and spin down to remove liquid from the cap.
10. Extract these samples following manufacturer instructions as outlined in the HDPCR SARS-CoV-2 Assay Instructions for Use, eluting into 50 µL.
11. Add 200 µL of Tube A into each of 3 wells/ cartridges on appropriate nucleic acid extraction system.
12. Add 200 µL of Tube B into each of 3 wells/ cartridges on appropriate nucleic acid extraction system.
13. Add 200 µL of Tube C into each of 3 wells/ cartridges on appropriate nucleic acid extraction system.

Testing Extracted Samples

Follow the HDPCR SARS-CoV-2 Assay Instructions for Use for testing of each concentration prepared as samples using your RUO instrument and corresponding run template file. All qualification specimens should be treated as samples. The qualification run must include standard controls (COV_Neg, COV_Pos, and NTC) and calibrators, as described in the Instructions for Use Running a New Test.

Expected Results

Results should be analyzed on ChromaCode Cloud as outlined in the Instructions for Use, but criteria for passing qualification specimens must be assessed by the end user according to the following Expected Results and Acceptance Criteria.

Replicates from Tube A contain a moderate concentration of COV_Pos (~100 copies/reaction,) and should be positive for N1, N2, and RNase P.

Replicates from Tube B contain a low (near LoD) concentration of COV_Pos (~10 copies/reaction) and should be positive for N1, N2, and RNase P.

Replicates from Tube C contain only COV_Neg and should be negative for N1 and N2, but positive for RNase P.

Acceptance Criteria

Moderate Positive Samples (Tube A): 100% (3/3) should be in agreement with expected results.

Low Positive Samples (Tube B): At least 66% (2/3) should be in agreement with expected results.

Negative Samples (Tube C): 100% (3/3) should be in agreement with expected results.

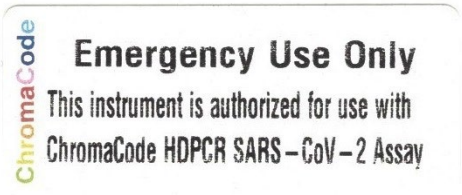
Successful qualification is required prior to the use of the following Real-Time PCR Systems with the HDPCR SARS-CoV-2 Assay for diagnostic testing:

Instrument
Applied Biosystems™ 7500 Fast
Applied Biosystems™ QuantStudio™ 5, 96-Well, 0.2 mL
Applied Biosystems™ QuantStudio™ 5 Flex, 384-Well
Applied Biosystems™ QuantStudio™ 7 Flex, 96-Well
Applied Biosystems™ QuantStudio™ 7 Flex, 384-Well
Applied Biosystems™ QuantStudio™ 12K Flex, 96-Well
Applied Biosystems™ QuantStudio™ 12K Flex, 384-Well

Appendix 2: RUO Applied Biosystems™ Real-Time PCR Systems Qualification Additional Label for RUO Applied Biosystems™Real-Time PCR Systems

Please print and place this label on the front panel of the instrument. Labels will also be proactively sent to sites using RUO instruments with the HDPCR SARS-CoV-2 Assay if self-printing is not preferred. If the instruments include labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the ChromaCode HDPCR SARS-CoV-2 Assay.

*Refer to Appendix 1: RUO Applied Biosystems Real-Time PCR Systems Qualification for instructions



HDPCR™SARS-CoV-2 Assay

Package Insert – Version 9, February 2022

IVD

COVID-19 Emergency Use Authorization Only
For *in vitro* diagnostic (IVD) Use | Rx Only

This Package Insert does not include the full Instructions for Use. See the electronic Instructions for Use (eIFU) for full details on running the SARS-CoV-2 Assay.

eIFU and the Healthcare Provider and the Patients Fact Sheets are available on ChromaCode.com through the Products Page for the HDPCR™SARS-CoV-2 Assay, the downloads page from ChromaCode Cloud Software, and from your Customer Service Representative free of charge.

Intended Use

The HDPCR SARS-CoV-2 Assay is a reverse transcription real-time polymerase chain reaction (qRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs as well as nasal aspirate and nasal wash from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The HDPCR SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The HDPCR SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principles of Procedure

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 nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, and nasal wash using the Thermo Scientific KingFisher Flex. The product includes the same N1 and N2 oligonucleotide primer and probe sequences for the detection of 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, effective 3/15/2020. 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. The N1 target is in the FAM channel, the N2 target is in the VIC channel, and the RNase P internal control (RNase P (IC)) is in the Cy5 channel.

Materials Provided and Storage

The product is available in a low volume test configuration (PN: 0683) or a High Throughput (HT) test configuration (PN: 0904 and PN: 0905). To run the HDPCR SARS-CoV-2 Assay in an HT configuration, both PNs 0904 and 0905 are required.
Control kits (PN: 0690) are available to be ordered and are required for both configurations.

Table 1. HDPCR SARS-CoV-2 Assay, 480 Tests (PN: 0683)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
HDPCR SARS-CoV-2 Subkit (480 Tests):	0682	1 Kit	N/A	2-8°C	-25 to -15°C
Enzyme Mix 03E	0688	5 Tubes	1050	2-8°C	-25 to -15°C
HDPCR SARS-CoV-2 Mix	0674	5 Tubes	450	2-8°C	-25 to -15°C
Reverse Transcriptase 01	0081	5 Tubes	120	2-8°C	-25 to -15°C
COV_A	0675	2 Tubes	80	2-8°C	-25 to -15°C
COV_B	0676	2 Tubes	80	2-8°C	-25 to -15°C
COV_C	0677	2 Tubes	80	2-8°C	-25 to -15°C
COV_D	0678	2 Tubes	80	2-8°C	-25 to -15°C
COV_E	0679	2 Tubes	80	2-8°C	-25 to -15°C

Table 2. HDPCR SARS-CoV-2 Box 1, HT Assay, 2592 Rxn (PN: 0904)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
Enzyme Mix 03E	0688	27 Tubes	1050	2-8°C	-25 to -15°C
HDPCR SARS-CoV-2 Mix	0674	27 Tubes	450	2-8°C	-25 to -15°C
Reverse Transcriptase 01	0081	27 Tubes	120	2-8°C	-25 to -15°C

Table 3. HDPCR SARS-CoV-2 Box 2, HT Calibrators, 36 Runs (PN: 0905)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
COV_A	0675	9 Tubes	80	2-8°C	-25 to -15°C
COV_B	0676	9 Tubes	80	2-8°C	-25 to -15°C
COV_C	0677	9 Tubes	80	2-8°C	-25 to -15°C
COV_D	0678	9 Tubes	80	2-8°C	-25 to -15°C
COV_E	0679	9 Tubes	80	2-8°C	-25 to -15°C

Table 4. HDPCR SARS-CoV-2 Controls, 20 Extraction Runs (PN: 0690)

Item	Part Number	QTY	Vol, µL	Shipping Condition	Storage Condition
COV_Neg	0681	20 Tubes	200	Dry ice	-25 to -15 °C
HDPCR SARS-CoV-2 Pos Ctrl/ Diluent, 4 ea:	0696	1 Kit	N/A	Dry Ice	-25 to -15 °C
COV_Pos	0680	4 Tubes	70	Dry ice	-25 to -15 °C
Diluent	0695	4 Tubes	200	Dry ice	-25 to -15 °C

Warnings and Precautions

There are no known hazardous substances included in the manufacture of the HDPCR SARS-CoV-2 Assay. Safety Data Sheets are available online at https://chromacodecloud.com/downloads or through ChromaCode Customer Support at customer.support@chromacode.com

Additional material or chemicals required for the use of the HDPCR SARS-CoV-2 Assay should be closely examined by the user. The user should carefully read all warnings, instructions or Safety Data Sheets provided by the supplier and follow the general safety precautions when handling biohazards, chemicals and other materials.

General Precautions

- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3 (b)(1), unless the declaration is terminated, or authorization is revoked sooner.
- Standard precautions and procedures should be taken when handling and extracting human samples.
- Standard precautions and procedures should be taken when using extraction instruments.

- Standard precautions and procedures should be taken when disposing of samples, extracted material and waste.
- Dispose of reagents according to local regulations.
- Do not use reagents after their recommended stability time frame.
- Do not mix reagent lots from different HDPCR SARS-CoV-2 Assay kits.
- Avoid contamination by following good laboratory practices, wearing proper personal protective equipment, segregating workflow, and decontaminating workspace appropriately.
- Ensure all consumables are DNase and RNase free.

See eIFU for full list of assay precautions.

Limitations

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may lead to erroneous results.

The performance of the HDPCR SARS-CoV-2 Assay was established using nasopharyngeal swab specimen type collected in UTM or VTM. Specimens should not be collected in saline. Anterior nasal swabs, oropharyngeal swabs, mid-turbinate nasal swabs as well as nasal aspirate, and nasal wash specimens are also considered acceptable specimen types for use with the HDPCR SARS-CoV-2 Assay but performance has not been established for these specimen types.

See eIFU for full list of assay limitations.

Trademarks

HDPCR™ is a trademark of ChromaCode, Inc.
All other product names and trademarks are the property of their respective owners.

Explanation of Symbols

The following symbols are present on the HDPCR SARS-CoV-2 Assay labels and kits.

Symbol	Definition
	Manufactured By
	Upper and Lower Storage Temperature Limitation
R_x	By Prescription Only
LOT	Lot Number
QTY	Quantity

Symbol	Definition
REF	Product Catalog Number
	Use by Date
IVD	In vitro Diagnostic Medical Device
	electronic Instructions for Use

Manufacturing/Distribution

Manufactured by
ChromaCode, Inc.
2330 Faraday Ave, Suite 100
Carlsbad, CA 92008 USA

Support

E-mail: technical.support@chromacode.com
Phone: +1 442-244-4370

Sales and Marketing

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