HDPCR™ SARS-CoV-2 Assay

Instructions for Use

IVD

COVID-19 Emergency Use Authorization Only

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

www.chromacode.com

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HDPCR™ SARS-CoV-2 Assay

In vitro diagnostic Real-Time PCR test for Coronavirus COVID-19

For Use With:		
Sample Types	**Extraction Platforms**	**PCR Platforms**
Nasopharyngeal swabs oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, nasal wash, and bronchoalveolar lavage (BAL) specimens	Roche MagNA Pure 24 Thermo Scientific™ KingFisher™ Flex	Applied Biosystems™ QuantStudio™ 7 (Fast Block) Applied Biosystems™ 7500 Fast Applied Biosystems™ QuantStudio™ 12K Flex(96-well Fast Block)

Table of Contents

Intended Use 4
Principles of Procedure 4
Assay Layout and Controls 5
Materials Provided and Storage 6
Materials Required but Not Provided 7
Warnings and Precautions 8
General Precautions 8
Specimen Collection and Storage 9
Running a New Test 10
Preparing to Run Assay on Instrument for the First Time 10
Registering an Instrument on ChromaCode Cloud 10
Download the Template Run File 10
Nucleic Acid Extraction 11
Create the Plate Layout Map 12
Prepare the Amplification Reaction Mix 12
Prepare COV_Pos Positive Control 13
Add Samples and Calibrators to Plate 13
Create a Run File and Start the Run 14
Data Export 14
Exporting .xls files from a QuantStudio 12K Flex Instrument 14
Exporting .eds files from a QuantStudio 7 or a 7500 Fast Instrument 14
Results Interpretation 15
Upload the Run Data to ChromaCode Cloud 15
Plate Quality Control 15
Sample Results Interpretation 18
Limitations 21
Performance Evaluation 23
Analytical Sensitivity: Limit of Detection (LoD) 23
Inclusivity: Analytical Sensitivity 24
Cross-Reactivity: Analytical Specificity 24
Clinical Performance Evaluation 25
Trademarks 26
Explanation of Symbols 27
Manufacturing and Distribution Information 27
Support 28
Sales and Marketing 28

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 human nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs as well as nasal aspirate, nasal wash, and bronchoalveolar lavage (BAL) specimens 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, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The 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-infections with other respiratory 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 positive 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 evaluated in combination 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 human nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirate, nasal wash, and bronchoalveolar lavage (BAL) specimens 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, 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 is not assessed.

The ChromaCode Cloud Software 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 1.

Table 1 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

Table 2 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 3 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 can 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 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 Extraction System
  - Roche MagNA Pure 24 Nucleic Acid Extraction System (Catalog Number 07290519001)
  - Thermo Scientific™ KingFisher™ Flex Extraction System (Catalog Number 5400630)
- One qRT-PCR Instrument
  - Applied Biosystems™ QuantStudio™ 7 Flex Software Version 1.3 (Fast Block: Catalog Number 4485698)
  - Applied Biosystems™ 7500 Fast Software Version 2.3 (Catalog Number 4351106)
  - Applied Biosystems™ QuantStudio™ 12K Flex Software Version 1.3 (96-well Fast Block: Catalog Number 4471088)

  Note: Instruments must be in current calibration per manufacturer specifications. qRT-PCR instrumentation must be calibrated for the following dyes: FAM, VIC, and Cy5.

- 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

Specific Consumables:

- Applied Biosystems™ MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plate with Barcode (Catalog Number 4483485)
- Applied Biosystems™ MicroAmp™ Optical Adhesive Film PCR/Real-time PCR Compatible (Catalog Number 4311971)
- For Roche MagNA Pure 24 Extraction System:
    - Roche MagNA Pure 24 Total Nucleic Acid Isolation Kit (Catalog Number 07658036001)
    - Roche MagNA Pure 24 Tip Park/Piercing Tool (Catalog Number 7345585001)
    - Roche MagNA Pure 96 Sealing Foil (Catalog Number 6241638001)
    - Roche MagNA Pure Filter Tips 1000 µL (Catalog Number 6241620001)
    - Roche MagNA Pure 24 Processing Cartridge (Catalog Number 7345577001)
- For Thermo Scientific™ KingFisher™ Flex Extraction System:
    - Applied Biosystems™ MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Catalog Number A42352)
    - KingFisher 96 well accessory kit from Macherey Nagel (Catalog Number 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

- The HDPCR SARS-CoV-2 Assay is for *in vitro* diagnostic use (IVD) only. Rx Only.
- For use under COVID-19 Emergency Use Authorization Only.
- Standard precautions and procedures should be taken:
    - when handling and extracting human samples;

- when using extraction instruments;
- disposing of samples, extracted material and waste.

- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or 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 and BAL specimens should be collected using standard procedures and recommendations from the collection device manufacturer. Swab specimens can be collected in UTM/VTM or equivalent.

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 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 be transported, maintain ≤-70°C on dry ice for overnight shipment.
- Extracted nucleic acids can be stored at ≤-70°C. If samples need 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.

Note: Any instrument running the HDCPR 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 the 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
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 4 Thermal Cycling Conditions for HDCPR 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**.

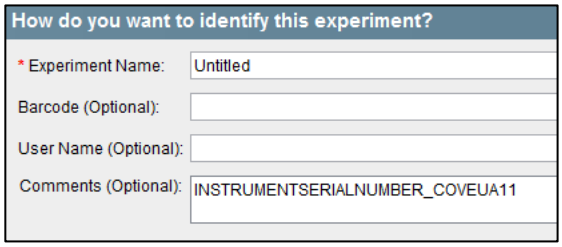
2. Download the .edt template run file for your instrument type and save the template file on your instrument.
   Note: Users need only download the template file and save to their instrument upon first use.

Table 5 Template Files for HDPCR SARS-CoV-2 Assay

Instrument	.edt filename
Applied Biosystems™ QuantStudio™ 7	COVEUA11_QS7_template
Applied Biosystems™ 7500 Fast	COVEUA11_7500Fast_template
Applied Biosystems™ QuantStudio™ 12K Flex	COVEUA11_QS12K_template

Applied Biosystems™ 7500 Fast Template File

1. Open the COVEUA11_7500Fast_template on your instrument.
2. Under the **Setup** tab on the left side, select **Experiment Properties**.
3. Add your instrument's serial number to the comments field where labeled **InstrumentSerialNumber**
   EX: 1234567890_COVEUA11



4. Select **File** on the Navigation tab and select **Save as Template**.
5. Name the file **COVEUA11_7500FAST_template.edt** and save in a preferred location.

Applied Biosystems™ QuantStudio™ 12K Flex and 7 Template File

1. Open the COVEUA11 template file on your instrument and proceed to running HDPCR SARS-CoV-2 Assay.

Nucleic Acid Extraction

Refer to Roche MagNA Pure 24 or 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:

- Pathogen 200 2.0 Protocol and the MagNA Pure 24 Total Nucleic Acid Isolation Kit (Product Number 07658036001) on the Roche MagNA Pure 24 System
- 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 five 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 (Table 6) at room temperature until no ice crystals remain:

Table 6 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 Enzyme Mix 03E and Reverse Transcriptase 01 five (5) times and spin to remove liquid from the cap.

3. Prepare the Amplification Reaction Mix in a 2 mL tube according to the following table, where n = the number of reaction wells to be run.

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

Table 7 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 COV_Pos and Diluent 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 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 to the plate layout map.
5. Add 5 µL of each sample to the wells in accordance to the plate layout map.
6. Add 5 µL of molecular grade water as the NTC in accordance to 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.

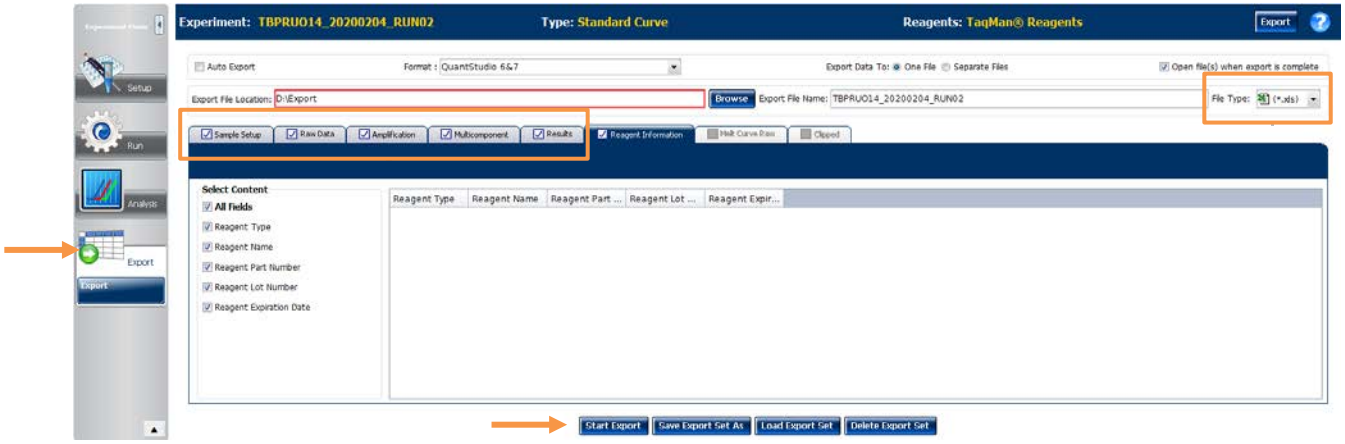
1. On instrument software, open the provided instrument specific template **SARS-CoV-2 template**
2. Ensure the Sample, Calibrator, and Control Names are correctly entered in software based on the Plate Layout Map.
3. Start the run in the software.

Data Export

Data must be exported from the thermal cycling instrument for upload onto ChromaCode Cloud for interpretation. The ChromaCode cloud accepts the .eds file format from the QuantStudio 7 and 7500 Fast—this file type does not require additional steps. The ChromaCode Cloud requires .xls file format from the QuantStudio 12K Flex. Please follow the steps below to export the .xls file format.

Exporting .xls files from a QuantStudio 12K Flex Instrument

1. Upon run completion, export the .xls run file by navigating to the **Export** tab, selecting .xls as **File Type**, and clicking **Start Export**.
   *Note: Ensure the **Sample Setup, Raw Data**, **Amplification**, **Multicomponent**, and **Results** checkboxes are clicked.*



Exporting .eds files from a QuantStudio 7 or a 7500 Fast Instrument

1. Upon run completion, navigate to **File**, then select **Save**. Proceed to save the .eds file where convenient.

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**.

4. Browse to find the desired run file (.eds for QuantStudio 7 and 7500 Fast and .xls for QuantStudio 12K Flex) 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.

The ChromaCode Cloud automatically assesses the plate QC status. This information is provided in a Plate Summary view and a Well Details page on the 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** (seen in Figure 1).

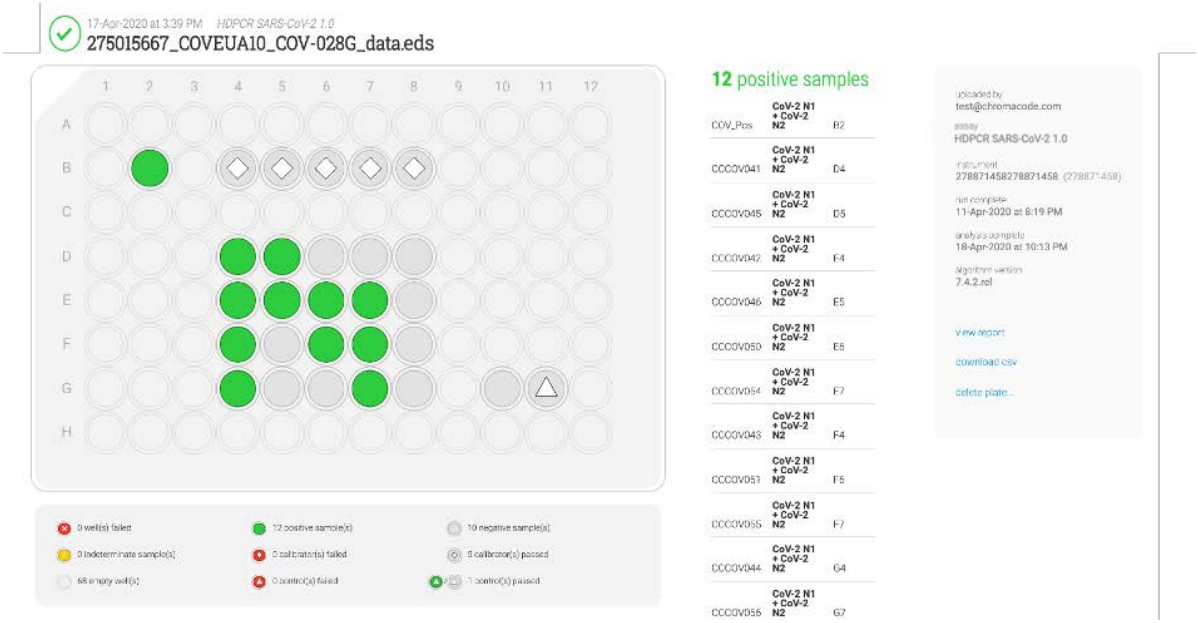


Figure 1 Plate Passing ChromaCode Cloud QC

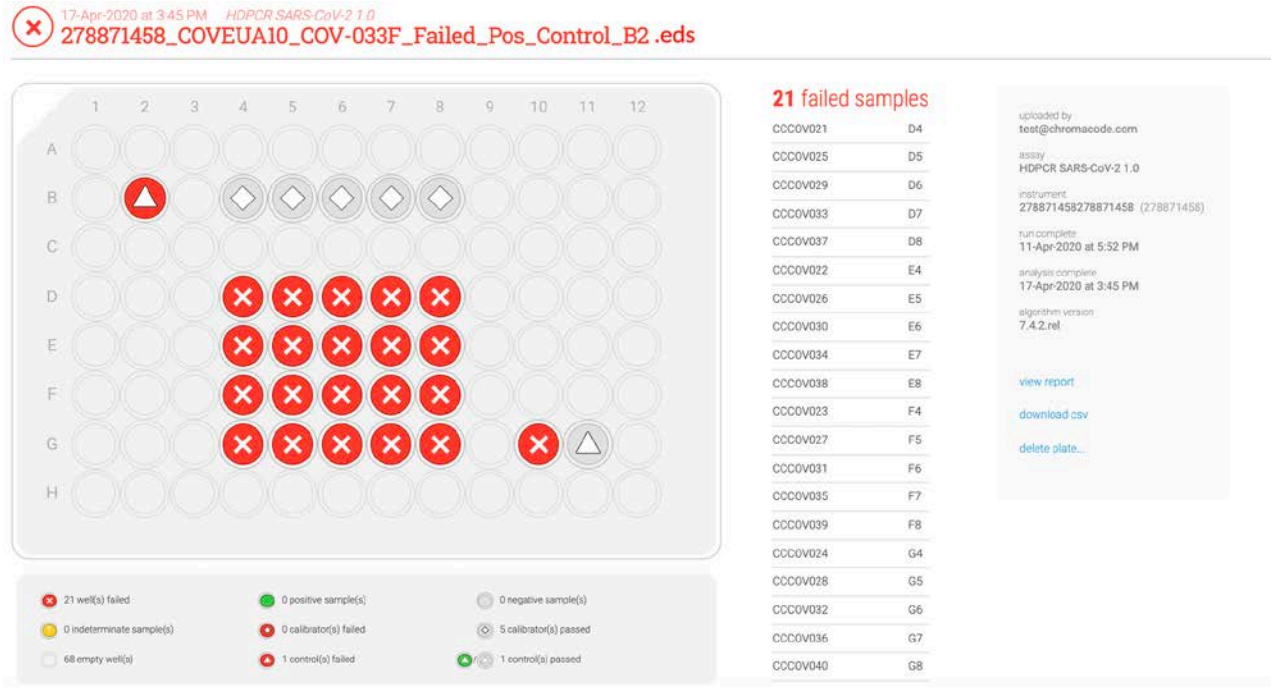


Figure 2 Plate Summary Page with Failing Plate QC

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 the 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 2). A plate that has passed plate QC will have a green check and the run name will be written in green text (Figure 1).

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 8 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 1) 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 9.

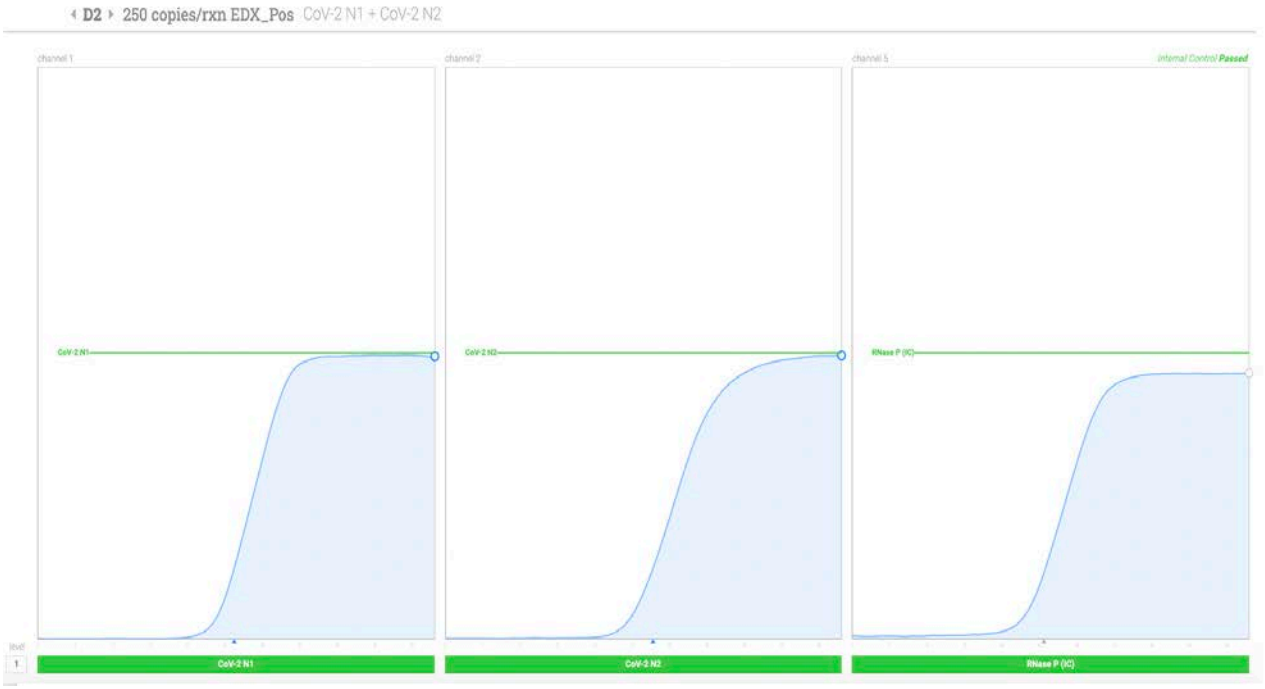


Figure 3 Well Details Page Containing Targets

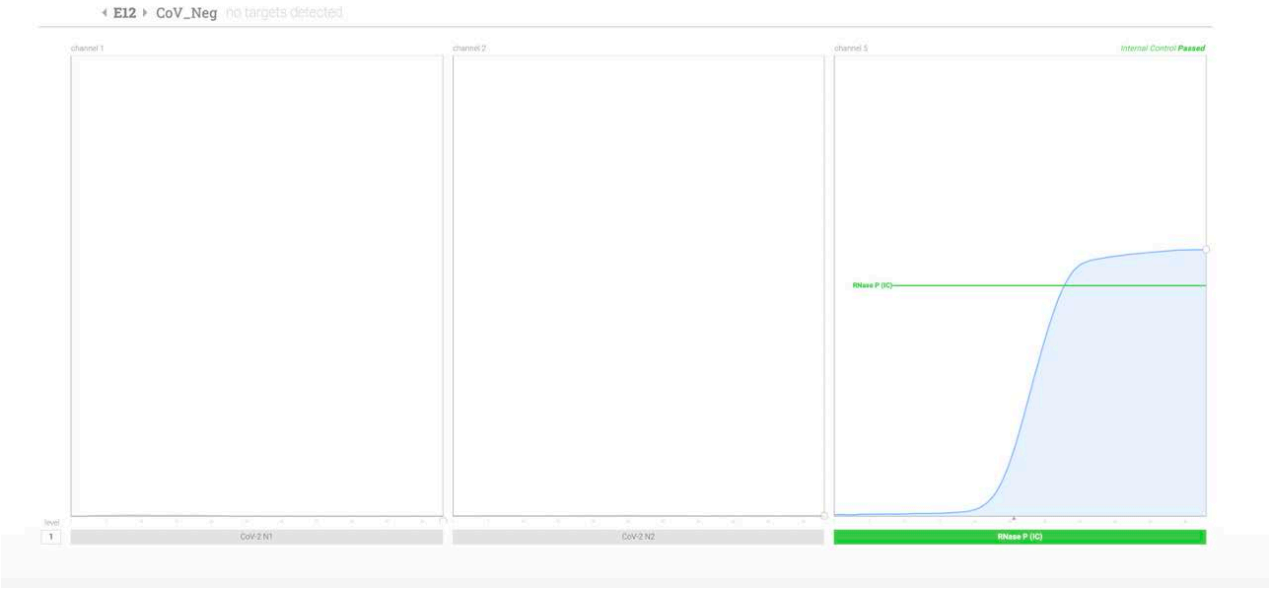


Figure 4 Well Details Page Containing Negative Sample

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 3). 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 4). 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 the 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

The following table outlines the expected results from the assay and potential recourse that must be taken.

Table 9 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 that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity test by Rx only.

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 VCM transport media. Nasal swabs, oropharyngeal swabs, mid-turbinate nasal swabs, nasal aspirate, nasal wash and BAL specimens are also considered acceptable specimen types for use with the HDPCR SARS-CoV-2 Assay but performance has not been established.

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

The HDPCR SARS-CoV-2 Assay performance was established using the Roche MagNA Pure 24 System with the Roche MagNA Pure 24 Total Nucleic Acid Isolation Kit and the Pathogen 200 2.0 Protocol with the ABI QuantStudio 7 and ABI 7500 Fast.

Performance on the ABI QuantStudio 12K Flex 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, 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 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.

Positive 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/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#2019-ncov.
However, to assist clinical laboratories running the HDPCR SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below:
A. Authorized laboratories[1] using the HDPCR SARS-CoV-2 Assay will 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 the HDPCR SARS-CoV-2 Assay will use the HDPCR SARS-CoV-2 Assay as outlined in the HDPCR SARS-CoV-2 Assay Instructions for Use. 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 perform the HDPCR SARS-CoV-2 Assay are not permitted.

C. Authorized laboratories that receive the HDPCR SARS-CoV-2 Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the HDPCR SARS-CoV-2 Assay will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and ChromaCode Inc. 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 the test must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

G. ChromaCode, Inc, authorized distributors, and authorized laboratories using the HDPCR SARS-CoV-2 Assay will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

1 The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

Performance Evaluation

The following data demonstrate the performance of the HDPCR SARS-CoV-2 Assay. All sample extractions for samples used 200 µL of specimen input eluting into 50 µL. The Roche MagNA Pure 24 Pathogen 200 2.0 Protocol was used with the Total Nucleic Acid Isolation Kit with qRT-PCR on the Applied Biosystems QuantStudio 7 and 7500 Fast instruments. The KingFisher MVP_Flex Protocol was used with the Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit with qRT-PCR on the Applied Biosystems QuantStudio 12K Flex

Analytical Sensitivity: Limit of Detection (LoD)

The LoD of the HDPCR SARS-CoV-2 Assay was determined using the Armored RNA Quant® SARS-CoV-2 control obtained from Asuragen (Catalog Number 52030) spiked into negative nasopharyngeal swab specimens in 2-fold dilution across the range (2000 to 125 copies/mL) in triplicate. The spiked specimens were extracted using the Roche MagNA Pure 24 Pathogen 200 2.0 Protocol with the Total Nucleic Acid Isolation Kit for qRT-PCR on the Applied Biosystems QuantStudio 7 and 7500 Fast instruments. The KingFisher MVP_Flex Protocol was used with the Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for qRT-PCR on the Applied Biosystems QuantStudio 12K Flex. For all extractions, 200 µL of spiked specimen input was eluted into 50 µL. The eluate was evaluated with the HDPCR SARS-CoV-2 Assay. The lowest copy to yield 3 of 3 N1 and N2 detections was 1000 copies/mL on the Applied Biosystems QuantStudio 7 and 7500 Fast and 250 copies/mL on the Applied Biosystems QuantStudio 12K Flex. The LoD was verified by testing 20 additional extraction replicates spiked at 1000 and 500 copies/mL for the Applied Biosystems 7500 Fast and the Applied Biosystems QuantStudio 7. The LoD was verified by testing 20 additional extraction replicates spiked at 500 and 250 copies/mL for the Applied Biosystems QuantStudio 12K Flex. The limit of detection for the HDPCR SARS-CoV-2 Assay for various instrumentation is in Table 10, assuming 100% extraction efficiency.

Results of LoD confirmatory testing demonstrated an assay LoD (when both N1 and N2 targets are detected for at least 95% of sample replicates) of 1000 copies/mL for both the Applied Biosystems 7500 Fast and the Applied Biosystems Quant Studio 7 systems and 250 copies/mL for the Applied Biosystems Quant Studio 12K system.

Table 10: Limit of Detection Confirmation Testing Summary

Instrument	Concentration (copies per mL) in extraction	*Concentration (copies per Reaction)	Detection Rate: CoV-2 N1	Detection Rate: CoV-2 N2
ABI 7500 Fast	1000	20	20/20	20/20
	500**	10	18/20	20/20
ABI QuantStudio 7	1000	20	19/20	20/20
	500**	10	18/20	20/20
ABI QuantStudio 12K	500	10	20/20	20/20
	250	5	20/20	19/20

* Assumes 100% extraction efficiency

**Preliminary LoD Estimation Studies showed at 250 copies/mL 1/3 N1 and 3/3 N2 targets were detected. At 125 copies/mL 1/3 N1 and 2/3 N2 targets were detected. Confirmatory testing was not performed at these concentrations.

Inclusivity: Analytical Sensitivity

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. The inclusivity analysis can be found in the referenced document found at the following URL: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

Cross-Reactivity: Analytical Specificity

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. The exclusivity analysis can be found in the referenced document found at the following URL: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

Clinical Performance Evaluation

The Clinical Evaluation of the HDPCR SARS-CoV-2 Assay was conducted using 30 negative nasopharyngeal swab specimens and 30 contrived positive samples. The contrived specimens were prepared by spiking negative nasopharyngeal specimens with varying concentrations of Armored RNA Quant® SARS-CoV-2 Control obtained from Asuragen (52030). Contrived specimens were extracted using the Roche MagNA Pure 24 Pathogen 200 2.0 Protocol with the Total Nucleic Acid Isolation Kit for qRT-PCR on the Applied Biosystems QuantStudio 7 and 7500 Fast instruments. The KingFisher MVP_Flex Protocol was used with the Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for qRT-PCR on the Applied Biosystems QuantStudio 12K Flex. For all extractions, 200 µL of contrived specimen input was eluted into 50 µL.

Twenty of the contrived reactive specimens were spiked with 1,000 copies/mL, and the remaining 10 were spiked in duplicate at 5,000, 25,000, 250,000, 2,500,000 and 25,000,000 copies/mL on the Applied Biosystems QuantStudio 7 and 7500 Fast instruments. Twenty of the contrived reactive specimens were spiked at 1000 copies/mL, and the remaining 10 were spiked in duplicate at 5,000, 10,000, 25,000, 50,000 and 500,000 copies/mL on the Applied Biosystems QuantStudio 12K Flex instrument. All instruments had 30 negative samples run in addition to contrived reactive specimens.

Table 11 Clinical Evaluation Data Summary on Applied Biosystems 7500 Fast

Spiked RNA Concentration (copies/mL)	SARS-CoV-2 N1	SARS-CoV-2 N2	Presumptive Positive
Negative	29/30	30/30	1/30*
1,000	20/20	20/20	0/20
5,000	2/2	2/2	0/2
25,000	2/2	2/2	0/2
250,000	2/2	2/2	0/2
2,500,000	2/2	2/2	0/2
25,000,000	2/2	2/2	0/2

Positive Percent Agreement: 30/30 = 100% (CI: 88.7-100%)
Negative Percent Agreement: 29/30 = 96.7% (CI: 83.3-99.4%)

* A second extraction of this specimen was negative for both N1 and N2

Table 12 Clinical Evaluation Data Summary on Applied Biosystems QuantStudio 7

SARS-CoV-2 Concentration (copies/mL)	SARS-CoV-2 N1	SARS-CoV-2 N2	Presumptive Positive
Negative	29/30	30/30	1/30*
1,000	20/20	20/20	0/20
5,000	2/2	2/2	0/2
25,000	2/2	2/2	0/2
250,000	2/2	2/2	0/2
2,500,000	2/2	2/2	0/2
25,000,000	2/2	2/2	0/2

Positive Percent Agreement: 30/30 = 100% (CI: 88.7-100%)
Negative Percent Agreement: 29/30 = 96.7% (CI: 83.3-99.4%)

* A second extraction of this specimen was negative for both N1 and N2

Table 13 Clinical Evaluation Data Summary on Applied Biosystems QuantStudio 12K Flex

SARS-CoV-2 Concentration (copies/mL)	SARS-CoV-2 N1	SARS-CoV-2 N2	Presumptive Positive
Negative	30/30	30/30	0/30
1,000	20/20	20/20	0/20
5,000	2/2	2/2	0/2
10,000	2/2	2/2	0/2
25,000	2/2	2/2	0/2
50,000	2/2	2/2	0/2
500,000	2/2	2/2	0/2

Positive Percent Agreement: 30/30 = 100% (CI: 88.7-100%)
Negative Percent Agreement: 30/30 = 100% (CI: 88.7-100%)

Trademarks

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
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
	Information

Manufacturing and Distribution Information

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