October 2020

Co-Dx

Logix Smart™ SARS-CoV-2 (genes RdRp/E)

For in vitro diagnostic use

REF COVID-K-002

Logix Smart™ SARS-CoV-2 (genes RdRp/E)
CO-DIAGNOSTICS, INC.

CE

CO-DIAGNOSTICS, INC. | 2401 Foothill Dr., Ste D, Salt Lake City, UT 84109 USA

IVD

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1 INTENDED USE

The Logix Smart SARS-CoV-2 (genes RdRp/E) test is a real-time RT-PCR multiplex test intended for the *in vitro* qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), targeting the genes RdRp in the polygene Orf1ab region and gene E of the virus genome, in lower respiratory tract samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva from individuals suspected of COVID-19.

Results are used for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in lower respiratory tract samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva 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. Many laboratories 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 combined with clinical observations, patient history, and epidemiological information.

The Logix Smart SARS-CoV-2 test is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

2 PRODUCT DESCRIPTION AND TEST PRINCIPLE

The Logix Smart SARS-CoV-2 (genes RdRp/E) test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test utilizing the Company's patented CoPrimer technology (Satterfield, 2014) (Poritz & Ririe, 2014). The two sets of SARS-CoV-2 CoPrimer (one set for gene RdRp and a second set for gene E) are designed to detect RNA from the SARS-CoV-2 in lower respiratory tract samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva from patients who are suspected of COVID-19.

Each **Logix Smart SARS-CoV-2 (genes RdRp/E) test** kit consists of the following components:

- Ready-to-use Master Mix, complete with RNaseP internal positive control to verify sample quality.
- Positive Control (PC), to verify the performance of the master mix.
- Nuclease-Free Water as a negative control, to verify the master mix is free of contamination.

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2.1 Principles of Operation

The test begins with the selection of the sample type, followed by a collection of the sample by a trained healthcare provider. The sample must be identified following the laboratory quality system and current regulation. The sample must be stored properly until testing in the same facility or shipping to the assigned laboratory.

The Logix Smart SARS-CoV-2 (genes RdRp/E) test kit assay is a multiplexed single-step real-time reverse transcription PCR test that can be broken down into the following 3 stages:

- Sample preparation
- Reverse transcription
- Polymerase chain reaction (PCR) with real-time monitoring.

The assay also includes an internal positive control (IPC) that acts as an extraction control to confirm the performance of the extraction.

The sample preparation for PCR requires the samples to be processed to break apart cells and viruses to expose the genetic material. For this process, a commercially available extraction system is used. In this process, the nucleic acids are isolated and purified from the lower respiratory tract fluids (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), or the upper respiratory tract fluids (e.g., nasopharyngeal and oropharyngeal swabs).

The purified nucleic acid is then plated with the Logix Smart SARS-CoV-2 master mix, 5 µl of each. The master mix is pre-mixed and contains the necessary components to perform both the reverse transcription and PCR and does not need to be prepared ahead of time by the user.

The plated reactions will then be put in the thermocycler using the following cycling conditions:

- 15 min at 45°C,
- 2 min at 95°C,
- 45 cycles x [3s at 95°C, 32s at 55°C].

The 15-minute step at 45°C is the reverse transcription step, where the cDNA is created from the RNA template. The 2 min at 95°C is to inactivate the reverse transcriptase and acts as the initial denaturation step for PCR, which is then followed by the thermocycling for the PCR.

During the PCR, the FAM labeled forward CoPrimer acts as both the forward primer and probe. During the annealing/extension phase of the PCR, the 5' nuclease activity of Taq polymerase degrades the CoPrimers portion that annealed to the targeted sequence, causing spatial separation between the fluorophore and the quencher, generating a fluorescent signal. With each cycle, additional fluorophore molecules

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are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at the end of each cycle by the real-time thermocycler, specifically the CoDx Box. See Table 1 for the components included in the test kit.

Table 1

Components Included in the Test Kit

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Brown	Logix Smart SARS-CoV-2 Master Mix	MM	COVID-MM-002	Proprietary blend of SARS-CoV-2 CoPrimer™ and PCR reagents	1x500µL (100 reactions) or 1x1250µL (250 reactions) or 1x25000 µL (5,000 reactions)
Red	Logix Smart SARS-CoV-2 Positive Control	PC	COVID-PC-002	Proprietary blend of SARS-CoV-2 synthetic templates	1x500µL (100 reactions) or 1x1250µL (250 reactions) or 1x25000 µL (5,000 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1x500µL (100 reactions) or 1x1250µL (250 reactions) or 1x25000 µL (5,000 reactions)

3 REAGENT STORAGE AND HANDLING

Do the following when storing and handling reagents:

- Check that the components of the kit arrive frozen. The **Logix Smart SARS-CoV-2 (genes RdRp/E) kit** is shipped on dry ice. If one or more of the components are not frozen upon receipt or are compromised during shipment, contact your distributor for assistance.
- Immediately store all components at or below -20°C to prevent degradation of reagents.
- Always work with each **Logix Smart SARS-CoV-2 (genes RdRp/E)** component on ice. Make aliquots, if necessary, to avoid multiple freeze/thaw cycles.
- If you work in an area prone to power outages, have a back-up generator for your freezer as well as a temperature data log to ensure that the **Logix Smart SARS-CoV-2 (genes RdRp/E)** test kit remains frozen at -20°C.

Note: Stability data for the product is currently being collected and results will be published, and new Instructions for Use updated to reflect the stability conditions.

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4 MATERIAL REQUIRED BUT NOT INCLUDED WITH THE TEST

See Table 2 for extraction systems required but not included with the test and Table 3 for thermocyclers validated but not included with the test.

Table 2

Extraction and Automation Systems Validated with the Test

Extraction Reagent		Automation Platform (If applicable)	Manufacturer	Sample Input Volume/Sample Elution Volume
Name	Cat. Number			
QIAamp Viral RNA Mini Kit (Qiagen)	52904 (50 extractions) 52906 (250 extractions)	N/A	Qiagen	200 µL / 60 µL
Sbeadex Viral RNA Purification kit (Biosearch Technologies)	NAP-40-026-04 (5000 extractions)	oKtopure High Throughput DNA extraction Robot (KBS-0009-001)	LGC Biosearch	200 µL / 60 µL
Viral DNA/RNA kit (CW Bio)	CW3123S, CW3123M, CWY070	N/A	CoWin Biosciences (CWBio)	200 µL / 60 µL
HighPrep Viral DNA/RNA kit (MagBio)	HPV-DR96	N/A	MagBio	200 µL / 60 µL

Table 3

Thermocyclers Validated but Not Included with the Test

Thermocycler Machine	Catalog Number	Manufacturer
CoDx Box	MIC-4	Co-Diagnostics, Inc.
Mic qPCR Cycler	MIC-4	BMS, Bio Molecular Systems
QuantStudio™ 5 Real-Time PCR System	A34322	Applied Biosystems (Thermo Fisher Scientific)
CFX 96 Touch Real-Time PCR Detection System	1855195	Bio-Rad

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4.1 Consumables Required but Not Provided

The following is a list of consumables required but not provided:

- Disposable powder-free gloves and lab coats
- Disposable pipette tips with filters
- 10% bleach or other appropriate cleaning solution that degrades nucleic acids.
- PCR plates or strip tubes for the thermocycler being used

4.2 Equipment Required but Not Provided

The following is a list of equipment required but not provided:

- Several micropipettes capable of pipetting volumes from 5 µL to 1000 µL
- A cold block or ice
- Vortex and centrifuge
- Class II Biosafety cabinet, ideally in a BSL-2 containment facility, for the extraction
- PCR workstation, for master mix plating and setup
- Appropriate thermocycler

5 WARNINGS AND PRECAUTIONS

WARNING!

Before performing any testing or running any patient sample, verify that all instruments have been properly installed, calibrated, and are well maintained. Do **not** use instruments with an outdated calibration.

As with any diagnostic or laboratory experiment, good laboratory practices for molecular biology is essential to the proper performance of the qPCR or any laboratory experiment. Attention should be taken to the procedures particular to the molecular diagnostics procedures. Due to the high sensitivity of **Logix Smart SARS-CoV-2 (genes RdRp/E)** and the qPCR technology, care should be taken while handling samples and materials while performing the assay to keep reagents and amplification mixtures free of contamination.

Users should do to the following:

- Use sterile pipette tips with filters.
- Use standard precautions when handling any patient samples, as they may contain infectious agents.

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- Store and extract positive materials (specimens, positive controls, and amplicons) separately from other reagents.
- Always use nuclease-free water, provided with this kit.
- Consult appropriate Safety Data Sheets (SDS) for safety. The SDS for the **Logix Smart SARS-CoV-2 (genes RdRp/E)** test kit is provided with the shipment. If not provided with the shipment, the SDS can be retrieved from Co-Diagnostics website at the link: http://codiagnostics.com/products/diagnostic-solutions/
- To prevent contamination, it is required to use Good Laboratory Practices for Molecular Biology, which requires a unidirectional workflow and the separation of negative and positive materials.
- Always use the most recent version of this document as more information is added with future studies. This can be downloaded for free at http://codiagnostics.com/resources/instructions-for-use/

6 SAMPLE COLLECTION, HANDLING, TRANSPORT, AND STORAGE

The sample selection, collection, storage, and handling play an essential part in the performance of nucleic acid assays. If the laboratory does not have internal procedures for selection, collection, storage, and handling of the patient specimen, this section provides some basic guidelines in case of need; however, laboratories should follow internal validation and procedures for sample selection, collection, transport, and storage, and any other handling procedures.

For more information, visit the CDC's and WHO's websites in the following addresses:

- CDC - https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html
- WHO - https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance

6.1 Lower Respiratory Tract Fluids

6.1.1 Bronchoalveolar lavage, or tracheal aspirate: collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack.

6.1.2 Sputum: have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack.

6.2 Upper Respiratory Tract Fluids

6.2.1 Nasopharyngeal swab AND oropharyngeal swab (NP/OP swab): use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs

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or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing viral transport media. NP and OP specimens should be kept in separate vials. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack.

Note: Nasopharyngeal swab: Insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions/ Swab both nasopharyngeal areas with the same swab.

6.2.2 Oropharyngeal swab (e.g., throat swab): swab the posterior pharynx, avoiding the tongue.

6.2.3 Nasopharyngeal wash/aspirate or nasal aspirate: collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack. Or collect the sample on a vial with virus transport media which does not demand refrigeration or cold chain transport, it may require validation by the laboratory.

6.3 Saliva

Collect 2-3 mL into a sterile, leak-proof, screw-cap container, or collect saliva up to the fill-line for screw-cap saliva collection kits. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on ice pack. For saliva collection kits, follow the manufacturer's instructions for storage and shipping conditions.

6.4 Sample Handling

Laboratory workers should wear appropriate personal protective equipment (PPE), which includes disposable gloves, laboratory coat/gown, and eye protection when handling potentially infectious specimens.

Clinical specimens from patients suspected or confirmed to be infected with COVID-19 should be conducted under a certified class II biosafety cabinet in a BSL-2 containment facility. More details are provided in the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (CDC, 2009) or the *WHO Laboratory Biosafety Manual* (WHO, 2004).

For specific instructions on the handling of clinical specimens for coronavirus disease 2019, see also the CDC's webpage for the *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019* (COVID-19) (CDC, 2020).

6.5 Sample Storage

It is recommended that all specimen types, be kept at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C. Repeated freezing

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and thawing of a specimen should be avoided. If a specimen is kept for retesting, it should be aliquoted in different tubes to avoid freezing and thawing cycles. The temperature in the storage areas should be monitored and recorded regularly to identify potential fluctuations. Domestic refrigerators/ freezers with wide temperature fluctuations are not suitable for the storage of frozen specimens (CDC, 2020).

6.6 Sample Shipping

Specimens known to be, or suspected of, containing SARS-CoV-2 that require shipment by air should be shipped on dry ice as a Biological Substance Category B, UN3373. International regulations, as described in the WHO *Guidance on Regulations for the Transport of Infectious Substances 2015-2016,* should be followed (CDC, 2020). If ground transportation is needed, the specimen should be shipped frozen overnight with enough ice to keep it frozen throughout transit. After the collection of the sample and transfer to the clinical lab, the sample will receive an entry into the laboratory system.

7 PROCEDURE

The World Health Organization recommends recording the full name, date of birth, contact information, and the time and date of collection of the patient sample. Additionally, the following information could also be collected:

- Symptoms, date of onset, duration of symptoms, contact with known COVID-19 cases (e.g., family member).
- Comprehensive travel history (dates, place, duration of visit, etc.).

7.1 Sample Preparation

The quality of the RNA from the extraction of the sample is essential to the performance of **Logix Smart SARS-CoV-2 (genes RdRp/E) kit**. The extraction protocol should be performed following the manufacturer's instructions.

7.1.1 Extraction of RNA with QIAamp® Viral RNA Mini Kit, cat. no. 52904/52906, Qiagen (manually or automated with QIAcube automated system)

The extraction can be performed following the manufacturer's instructions using 140 µL of the sample, and an elution using 60 µL of buffer AVE. For additional sensitivity, load up to 200 µL of patient sample, and increase the volume of Buffer AVL from 560 µL to 800 µL. To ensure the removal of residual wash buffer from the sample prior to elution, an additional centrifugation step (see extraction procedure) using a new collection tube is required.

Due to the mucoid and mucopurulent, and therefore, viscous nature of sputum specimen a pre-processing of the sample is recommended before

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extraction. A protocol provided by the CDC and evaluated for COVID-19 for the processing of sputum samples is available by the CDC in the following link: https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf (CDC, 2020). It is important to note that this processing should only be done in conjunction with the QIAamp Viral RNA Mini Kit.

Incubate the sample, mixing occasionally, at room temperature until the sample is liquified, which can take up to 30 minutes. Use the liquified sample for downstream nucleic acid extraction, following the extraction system manufacturer's guidelines. Retain any residual liquified sample at -70°C.

7.1.2 Extraction of RNA with sbeadex Viral RNA Purification Kit, cat. no. NAP-40-026-04, LGC Biosearch using the oKtopure™ High Throughput DNA Extraction Robot, cat no. KBS-0009-001, LGC Biosearch.

7.1.3 The following is extraction information of sputum or saliva samples:

- Sputum samples were collected directly with a 50 mL conical tube, then diluted 1:8 with 10% TE buffer and vortexed.
- The 2 mL saliva samples were collected with the Spectrum Solution DNA Collection Device. Then they were diluted with 1.5mL of CV3 Spectrum Chemistry transport media, and further diluted with 10% TE buffer and vortexed for a final 1:3 dilution.
- Preparation of bead/binding mixture
- Each plate requires combining 46.1mL of binding buffer SB and 2.9 mL of sbeadex particles for a single oKtopure run.

7.1.4 Protocol for a Single 96 Well Plate

7.1.4.1 In a fresh oKtopure 96 well sample plate, 200 µL of sample was added into each well. If there were remaining wells of the plate not being used for a sample, 200 µL of nuclease free (NF) water was added into the wells.

7.1.4.2 Once all wells were filled with either sample or NF water, the 96 well plate was securely placed inside the oKtopure.

7.1.4.2.1 We reference the oKtopure software for the proper placement/location of reservoirs and 96 well plates for each in the following steps below.

7.1.4.3 30 mL of lysis buffer was measured into a clean reservoir, then securely placed into the oKtopure.

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7.1.4.4 200 µL of lysis buffer was then added to each well by the oKtopure.

7.1.4.4.1 The sample and lysis buffer were mixed (by hand pipetting) before adding the bead mixture.

7.1.4.5 Once the lysis step was completed, 48 mL of bead/binding mixture was measured into a clean reservoir.

7.1.4.6 340 µL of bead/binding mixture was then added to each well by the oKtopure.

7.1.4.7 Once completed, the 96 well sample plate was moved to the appropriate position, and the bead/binding mixture in the reservoir was removed. Then the following items were collected: 4 clean reservoirs, 1 lysis waste plate (96 square welled plate), and a 96 well elution plate (destination plate).

7.1.4.8 Both the lysis waste plate and the 96 well elution plate were placed in the appropriate position.

7.1.4.9 The following volumes of Buffer were added into each reservoir:

7.1.4.9.1 BN1 (B1)- 43.2mL, TN1 (B2)- 34.6mL, TN2 (B3)- 53.3mL, Elution Buffer- 7.2mL.

7.1.4.10 The template run file was verified (200 uL sbeadex) and the extraction was started.

7.1.4.11 The oKtopure add the following volumes of buffer into each well:

7.1.4.11.1 BN1- 300 µL, TN1- 240 µL, TN2- 370 µL, Elution Buffer- 60 µL.

7.1.4.11.2 Between adding the buffers, the oKtopure mixed the bead/buffer mixture to resuspend the beads.

7.1.4.12 Once completed the samples were either kept on ice or used immediately to run on PCR. Extra extracted material was stored at -80°C.

7.1.5 Manual extraction of RNA using the Viral DNA/RNA kit, cat no. CW3123S/ CW3123M/ CWY070, CWBio

The manufacturer's instructions should be followed with a 200 µL of sample, and a 60 µL elution volume.

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7.1.6 Manual extraction of RNA using the HighPrep Viral DNA/RNA Kit, cat no. HPV-DR96, MagBio

The manufacturer's instructions should be followed with a 200 µL of sample, and a 60 µL elution volume.

Wash buffers used in the extraction kit contain ethanol. It is important to eliminate any traces of ethanol before elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

7.2 Logix Smart SARS-CoV-2 Reagent Setup

Perform the following steps when setting up the Logix Smart SARS-CoV-2 Reagent:

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by molecular grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.
- Vortex all **Logix Smart SARS-CoV-2** Master Mix, Positive Control (PC), nuclease-free water (used as a no template control or NTC), and sample tubes for 3 seconds and briefly spin down before using to ensure properly mixed reagents and to remove any condensation or residue from the lids.
- Thaw all reagents and samples on **ice**, or a cold block, before starting setup.

7.3 Reaction Set Up

7.3.1 Every reaction setup should include enough reaction wells for the number of patient samples and at least one positive control and one NTC (# patient samples + 2 = total reaction wells needed), (e.g., 5 patient samples to test + 1 PC well + 1 NTC well = 7 total reaction wells.

7.3.2 All pipetting should be done on ice, if possible. Pipetting of PC and sample elution is recommended to be done in a separate area, or at a separate time, from Master Mix and NTC. Change pipette tips in-between patient sample elution and change pipette tips after pipetting each component. Pipet the PC last, if possible, to avoid contamination events.

7.3.3 Pipet 5 µL of Master Mix into each well being used in an appropriate optical plate or optical reaction tube (example: CoDx Box real-time PCR instrument uses 48-well reaction tubes).

7.3.4 Pipet 5 µL of the patient sample (elution from nucleic acid extraction) or 5 µL of a control (NTC and PC) to the appropriate well(s). At least one positive and one NTC control must be included in each run.

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7.3.5 Seal the reaction plate with an optical adhesive film or the reaction tubes with appropriate lids.

7.3.6 Place plate or tubes into the real-time PCR instrument in the correct orientation and start the run.

7.4 qPCR Instrument Setup for the CoDx Box

7.4.1 Contact the Laboratory 801-438-1036 ext. 03 or at www.co-dx.com/contact/ for the template file for download for use with the CoDx Box. The template file comes pre-programmed with the PCR instrument setup described in the section below.

7.5 qPCR Instrument Setup

7.5.1 Define the settings displayed in Table 4.

Table 4

Settings to Define

Item	Setting
Reaction Volume	10 µL
Ramp Rate	Default
Passive Reference	None

7.5.2 Program PCR instrument with the cycling conditions in Table 5.

Table 5

Cycling Conditions

Stage	Cycles	Temperature	Time
Reverse Transcription	1	45˚C	15 minutes
Initial Denature	1	95˚C	2 minutes
Amplification	45	95˚C	3 seconds
		55˚C	32 seconds

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7.5.3 Define the fluorescence detectors (dyes) as displayed in Table 6.

Table 6

Fluorescence Detector Definitions

Target	Detector Name	Reporter	Quencher
SARS-CoV-2 (*RdRp gene*)	SARS-CoV-2 *RdRp* gene	FAM™	BHQ® - 1
SARS-Co-2 (*E gene*)	SARS-CoV-2 *E* gene	CAL Flour® Orange 560	BHQ® - 1
Human RNaseP specific DNA (IPC)	RNaseP	CAL Flour® Red 610	BHQ® - 2

- When the run is finished, ensure that the run file is saved.

8 DATA ANALYSIS

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.

Verification and validation studies performed for Logix Smart™ SARS-CoV-2 (genes RdRp/E) (Catalog number COVID-K-002) were conducted following Good Laboratory Practices for Molecular Biology assays (Viana & Wallis, 2011). If these conditions are not met, the performance will show higher variability due to user errors while experimenting.

8.1 Analysis Settings

The analysis parameters on the CoDx Box or Mic qPCR Cycler should be set to the following, but after every run, the settings for the green channel (monitoring for SARS-CoV-2 *RdRp* gene), the yellow channel (monitoring for SARS-CoV-2 *E* gene), and the orange channel (monitoring for RNaseP (IPC)), should be verified to match the following:

- Check the box to “Auto Set Threshold”
- “Method” should be set to Dynamic
- “Threshold Level” should be set to 0.100.
- “Threshold Start” should be set to 1.00

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- "Ignore Cycles Before" should be set to 5.
- "Exclusion" should be set to Extensive
- "Fluorescence Cutoff Level" should be set to 5.0%
- "Initial Y-Axis Scale" should be set to Linear
- Check the box to "Auto Generate Analysis"

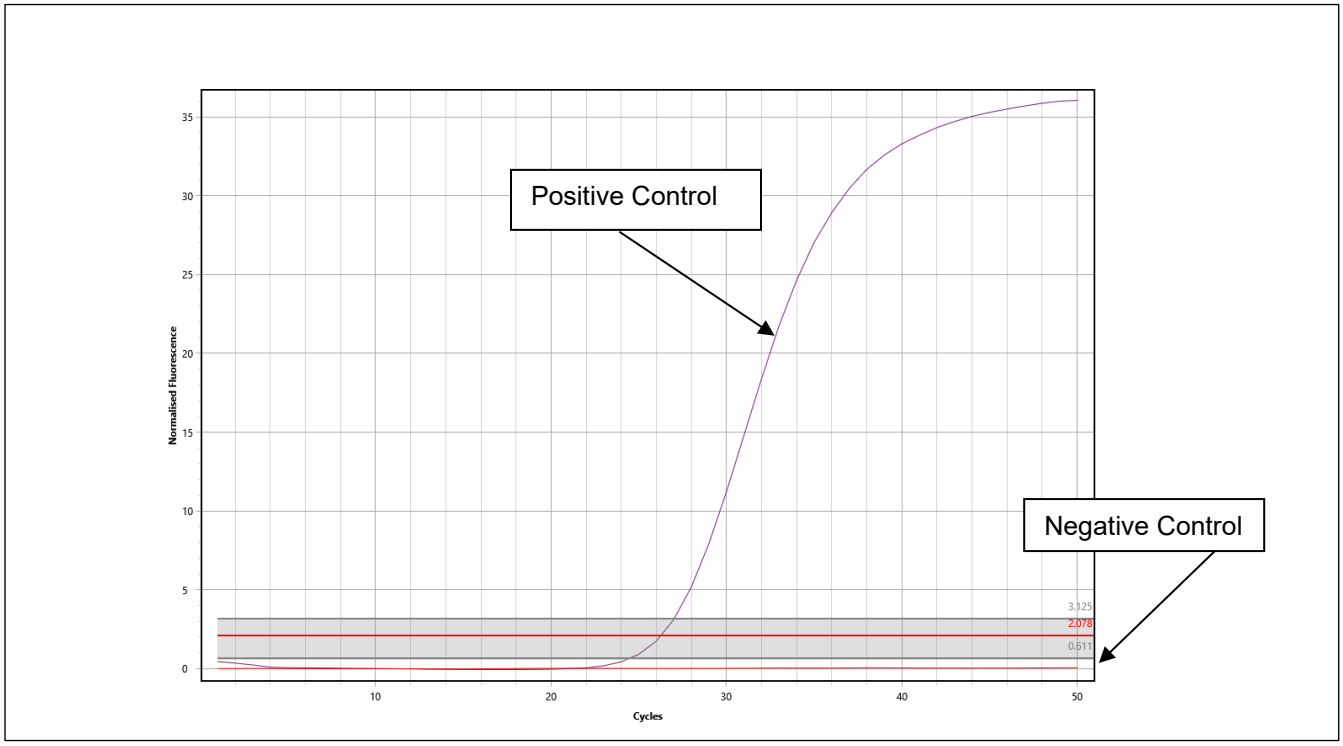
For other thermocyclers, follow the manufacturer's instructions for setting an appropriate threshold.

8.2 Positive Controls

Highlight the positive control reaction well. Each positive control should show an amplification curve for the SARS-CoV-2 *RdRp* marker in the FAM channel, SARS-CoV-2 *E-gene* marker in the CF560 channel, and amplification of the internal positive control for RNaseP (IPC) in the CF610 channel. A positive amplification curve looks similar to the purple curve in Figure 1 and should have a Cq value below 40 cycles.

Figure 1

Positive Control (PC) and No Template Control (NTC) Signals for Logix Smart SARS-CoV-2 (genes RdRp/E)



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8.3 Negative Control

Next highlight the negative control. The results of the negative control should show no amplification, specifically with a Cq value less than 40. An example of no amplification can be seen in Figure 1, as the red line, which is below the threshold area. The threshold area is the grey band with the red line.

8.4 The Validity of the Diagnostic Test Runs

Check to see that both the positive and no template control have passed.

8.4.1 The control conditions displayed in Table 7 must be met.

Table 7

Required Control Conditions

Control Type	Control Name	Purpose of Control	SARS-CoV-2 *RdRp* FAM channel	SARS-CoV-2 *E-gene* CF560 channel	Internal Positive Control (RNaseP) CF610 channel
SARS-CoV-2 Positive Control	SARS-CoV-2 *RdRp* (FAM™)	Verifies the performance of the master mix	+	+	+
	SARS-CoV-2 *E* (CF®560)				
	RNaseP (IPC) (CF®610)				
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-	-

- If controls pass, interpret the sample results.

8.4.2 Invalid Diagnostic Test Run

If any of the controls fail, the run is invalid. Document the run and initiate troubleshooting.

If Internal Positive Control (RNaseP) fails an investigation should be initiated to eliminate possible splashing, pipetting error, or any other laboratory error.

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8.5 Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes:

- Positive
- Negative
- Invalid

A **Positive** result will show an amplification curve or cycle threshold value for SARS-CoV-2 *RdRp/E* at or below 40 cycles. Amplification curves greater than 40 cycles for SARS-CoV-2 are in the uncertainty zone. The presence of a curve, with a Cq at or below 40 cycles, for a sample for the SARS-CoV-2 *RdRp/E*, indicates a positive result. The amplification of the RNaseP (IPC) shows that the extraction was successful.

A **Negative** result will show no amplification for SARS-CoV-2 *RdRp/E*; occasionally amplification greater than 40 cycles may occur in SARS-CoV-2 *RdRp/E* or RNaseP channels. Any amplification curves greater than 40 cycles are in the uncertainty zone and possibly below the limit of detection. New run of the same sample or run of another sample of the patient in the same of following days should be considered. The absence of a curve for SARS-CoV-2 *RdRp/E* indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

An **Invalid** result refers to situations when any of the controls fail. See troubleshooting. See Table 8.

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Table 8

Interpretation of Results for SARS-COV-2 detection with Logix Smart SARS-CoV-2

	Sample Result			Logix Smart™ SARS-CoV-2 Positive Control	No Template Control (NTC) (Master Mix + Water)	Interpretation of Results
	SARS-CoV-2 RdRp (FAM™)	SARS-CoV-2 E-gene (CF®560)	RNaseP (IPC) (CF®610)			
Instrument Reading	+	+	+	+	-	SARS-CoV-2 RNA +
	-	+	+	+	-	SARS-CoV-2 RNA +
	+	-	+	+	-	SARS-CoV-2 RNA +
	-	-	+	+	-	SARS-CoV-2 RNA -
	Any Result (+/-)		-	+	-	INVALID: See Troubleshooting
			+	-	-	
			+	+	+	

Amplification before 40 cycles is considered a positive reading (+). Amplification after 40 cycles is considered a negative reading (-). When possible, always check the medical history and/or symptoms to match the result before starting treatment.

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9 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Logix Smart™ SARS-CoV-2 (genes RdRp/E)** test kit**,** even if the recommended steps for troubleshooting solves the issue. To give feedback please fill out the Customer Feedback Form by visiting codiagnostics.com/contact/feedback/

9.1 Stability

Real-time, accelerated shelf-life, and in-use stability studies are currently under testing. Currently, the expiration date of this product has been established as 12 months.

Always use the most recent version of this document for updates as more stability information will be added when studies are completed.

9.2 User Errors

Polymerase Chain Reaction (PCR) Assay is a technique that uses temperature cycling, and a DNA polymerase to amplify a single or a few copies of a segment of DNA or RNA. Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel.

The user needs to have some molecular biology experience and be familiar with the proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection. Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment must have calibration up to date for the pipettes and thermocyclers, when applicable.

A 90-minutes online training for Good Laboratory Practices for Molecular Genetics Testing (CDC, 2017) is available at the CDC website at the following link https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html

9.3 Invalid Results

9.3.1 Logix Smart SARS-CoV-2 Positive Control not Amplifying

9.3.2 No amplification from the PC could be the result of one or multiple factors, such as the following:

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,

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- **Logix Smart SARS-CoV-2 Master Mix** or **Logix Smart SARS-CoV-2 Positive Control** degradation (a result of reagents being at temperatures above -20°C for an extended period),
- Use of expired reagents,
- or the wrong reagents being used.

Without further evidence, the run should be considered invalid and the user should re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the patient samples may need to be re-extracted. If failure of the positive control happens a third time after re-extraction and re-amplification, open a new **Logix Smart SARS-CoV-2 Positive Control** or **Master Mix**, and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 02 or contact us at www.co-dx.com/contact/.

9.3.3 RNaseP (IPC) not amplifying in patient samples

9.3.4 No amplification from the RNaseP channel could be the result of one or multiple factors, such as the following:

- Not enough nuclear material in the patient sample,
- PCR inhibitors such as ethanol and heparin,
- the extraction was performed incorrectly,
- or the extraction kit used is not compatible or has a step that eliminates RNaseP DNA.

Note: Positive amplification in the SARS-CoV-2 channel indicates a positive result despite the lack of concurrent amplification in the IPC channel. The IPC amplification is dependent on the presence of human genomic DNA (gDNA) in the extraction sample, the amount of which is governed by the type of the patient sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g., CSF, urine, cell lysates, etc.) may not contain the human RNaseP gene.

If IPC (CF610 channel) shows a negative result while SARS-CoV-2 *RdRp/E* (FAM/CF560) channel(s) shows positive result an internal investigation should be initiated.

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In the investigation, the following two possible scenarios should be evaluated:

- The positive result for SARS-CoV-2 *RdRp/E* (FAM/CF560) channel(s) is a true positive while the IPC is negative due to the lack of human RNaseP gene in the sample (absence of human cells in the sample).
- The amplification of SARS-CoV-2 *RdRp/E* (FAM/CF560) channel(s) is a false positive result while the IPC (CF610 channel) is negative due to testing/human errors potentially caused by mix-ups during plating and pipetting, refraction anomalies in the solution or any other cause for false positives.

Fail of any of the controls may indicate that the sample extraction or sample collection have failed. In this scenario a new extraction should be performed, if the IPC persists to be negative with negative SARS-CoV-2 channel the result should be reported as INVALID with "NEW SAMPLE COLLECTION NEEDED" request.

If the cause for an error is unclear, contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 02 or contact us at www.co-dx.com/contact/.

9.3.5 No Template Control showing amplification

Amplification of SARS-CoV-2 *RdRp/E* in the No Template Control indicates contamination of one or more of the reagents, incorrect placement of plate or tube into the real-time PCR instrument, or pipetting errors.

The results should be interpreted as invalid and re-testing by re-amplification should be performed. If the NTC fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the patient samples may need to be re-extracted. If failure of the NTC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, the run should be interpreted as invalid. Please contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 02 or at: www.co-dx.com/contact/.

10 LIMITATIONS

Note the following limitations:

- Strict compliance with this document is required for optimal results. Always use the most recent version of this document. This can be downloaded for free at co-dx.com/resources/instructions-for-use/

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- The use of this product is to be limited to trained and instructed personnel in real-time PCR techniques and IVD procedures.
- Good laboratory practices are essential for the proper performance of this assay. It is also recommended that upon receipt of reagents that a test run be performed to check the performance of the reagents before testing on patient samples.
- Appropriate specimen collection, transport, storage, and processing procedures are required for optimal results.
- Do not use the **Logix Smart SARS-CoV-2 (genes RdRp/E)** kit components directly on the specimens collected. Perform an appropriate nucleic acid extraction before using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the COVID-19, genome covered by this test kit may fail to detect the presence of the pathogens.
- As with any diagnostic test, results of the **Logix Smart SARS-CoV-2 (genes RdRp/E)** kit are to be interpreted with consideration of all clinical and laboratory findings.

11 ANALYTICAL EVALUATION

The analytical evaluation of performance was completed with contrived samples produced by spiking in SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI Resources, catalog number NR-52287) in a negative clinical matrix of mainly sputum, bronchoalveolar lavage (BAL), nasopharyngeal fluid, and nasal swab samples acquired from Discovery Life Sciences or donations.

11.1 Precision

The precision was performed over 10 days with 2 shifts run per day. Samples were prepared by spiking SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI Resources, catalog number NR-52287) reference material into saliva and then extracted. The concentrations used are identified as [Normal] = 300 copies/reaction (copies/ µL) and [Low] = 50 copies/reaction (10 copies/µL). The average Cqs between days should be less than or equal to 2.0 cycles with variance lower the 5% and p-value lower than 0.05. Results were found within the acceptance criteria. See Table 9 and Table 10.

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Table 9

p-Value in ANOVA for Precision Study of Logix Smart SARS-CoV-2

	p-Value (Days)
15428 (Green Channel)	
COVID [Normal]	7.82E-24
COVID [Low]	1.93E-19
26269 (Yellow Channel)	
COVID [Normal]	6.03E-26
COVID [Low]	1.57E-14

Table 10

Combined Precision Results

	Cq Average	SD	Call Rate	CV%	Marker Detection Rate (%)	Kit Detection Rate (%)
15428 (Green Channel)						
COVID [Normal]	31.71	1.15	172/172	3.63	100%	100%
COVID [Low]	34.02	1.25	169/172	3.67	98.25%	100%
26269 (Yellow Channel)						
COVID [Normal]	31.30	0.99	172/172	3.17	100%	100%
COVID [Low]	33.73	1.06	171/172	3.15	99.42%	100%

11.2 Limit of Detection (LoD) – Analytical Sensitivity

Limit of Detection (LoD) is the lowest concentration of analyte that is detected at a rate of no less than 95%. The experiment was performed using SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI Resources, catalog number NR-52287) which was spiked into sputum or samples after the lysis step of the specific extraction kit to prevent degradation of the RNA before the lysis. The following extraction kits were used for LoD testing: QIAamp Viral RNA Mini Kit (Qiagen, CAT#52906), HighPrep Viral DNA/RNA Kit (MagBio, CAT#HPV-DR96), and the Viral DNA/RNA Kit (CW Bio, CAT#CW3126M). Additionally, the sbeadex Viral RNA Purification Kit was run on the oKtopure (Biosearch Technologies, CAT#NAP-40-026-04). The LoD was evaluated for each kit individually. After the extraction process, the extracts were then tested using the Logix Smart SARS-CoV-2 test kit protocol. The LoD was then confirmed by performing 20 replicate extractions at the LoD concentration for each kit. See Table 11.

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Table 11

Genomic RNA strain SARS-CoV-2 (isolate USA-WA1/2020) Detection Rate

QIAamp Viral RNA Mini Kit (Qiagen)				HighPrep Viral DNA/RNA Kit (MagBio)			
SARS-CoV-2 Concentration in Sample (copies/µL)	# of Samples	# of Detected	Detection Rate (%)	SARS-CoV-2 Concentration in Sample (copies/µL)	# of Samples	# of Detected	Detection Rate (%)
8	8	8	100%	**6**	16	16	100%
6	8	8	100%	**4**	16	16	100%
4	8	8	100%	**2**	16	16	100%
2.5	16	16	100%	**1**	32	32	100%
2	8	8	100%	**0.8**	14	16	88%
1	23	24	96%	**0.6**	16	16	100%
0.8	16	16	100%	**0.5**	15	16	94%
0.5	13	16	81%	**0.4**	12	16	75%
0.2	5	16	31%	**0.1**	4	16	25%
Viral DNA/RNA Kit (CW Bio)				**Sbeadex Viral RNA Purification Kit (LGC Biosearch Technologies)**			
SARS-CoV-2 Concentration in Sample (copies/µL)	# of Samples	# of Detected	Detection Rate (%)	SARS-CoV-2 Concentration in Sample (copies/µL)	# of Samples	# of Detected	Detection Rate (%)
8	24	24	100%	8	32	32	100%
6	24	24	100%	6	32	32	100%
4	24	24	100%	4	31	32	97%
3	39	40	98%	3	16	16	100%
2	24	24	100%	2	24	32	75%
1.5	44	48	92%	1.5	16	32	50%
1	19	32	59%	1	14	16	88%
0.8	27	48	56%	0.8	11	16	69%
0.4	19	48	40%	0.4	10	16	63%
0.1	5	48	10%	0.1	1	16	6%

After those runs were completed, the lowest concentration with at least a 95% detection was then used for the verification of LoD runs. If a 95% detection rate was not achieved, the concentration was increased until at least a 95% detection rate was achieved. See Table 12.

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Table 12

Confirmation of the LoD

QIAamp Viral RNA Mini Kit (Qiagen)					
Thermocycler	**Concentration (copies/µL)**	**Sample Matrix**	**# Positive**	**Total Samples**	**Detection Rate**
CoDx Box	**0.8**	Saliva	20	20	100%
		Sputum	20	20	100%
MIC	**0.8**	Saliva	20	20	100%
		Sputum	20	20	100%
QuantStudio 5	**0.8**	Saliva	20	20	100%
		Sputum	20	20	100%
CFX96	**0.8**	Saliva	20	20	100%
		Sputum	20	20	100%
HighPrep Viral DNA/RNA Kit (MagBio)					
Thermocycler	**Concentration (copies/µL)**	**Sample Matrix**	**# Positive**	**Total Samples**	**Detection Rate**
CoDx Box	**1.0**	Saliva	20	20	100%
		Sputum	20	20	100%
MIC	**1.0**	Saliva	20	20	100%
		Sputum	20	20	100%
QuantStudio 5	**1.0**	Saliva	20	20	100%
		Sputum	20	20	100%
CFX96	**1.0**	Saliva	20	20	100%
		Sputum	20	20	100%
Viral DNA/RNA Kit (CW Bio)					
Thermocycler	**Concentration (copies/µL)**	**Sample Matrix**	**# Positive**	**Total Samples**	**Detection Rate**
CoDx Box	**2.0**	Saliva	20	20	100%
		Sputum	20	20	100%
MIC	**2.0**	Saliva	20	20	100%
		Sputum	20	20	100%
QuantStudio 5	**2.0**	Saliva	20	20	100%
		Sputum	20	20	100%
CFX96	**2.0**	Saliva	20	20	100%
		Sputum	20	20	100%
Sbeadex Viral RNA Kit (Biosearch Technologies)					
Thermocycler	**Concentration (copies/µL)**	**Sample Matrix**	**# Positive**	**Total Samples**	**Detection Rate**
CoDx Box	**6.0**	Saliva	20	20	100%
		Sputum	20	20	100%
MIC	**6.0**	Saliva	20	20	100%
		Sputum	20	20	100%
QuantStudio 5	**6.0**	Saliva	19	20	95%
		Sputum	19	20	95%
CFX96	**6.0**	Saliva	19	20	95%
		Sputum	19	20	95%

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The Limit of Detection (LoD) for Logix Smart SARS-CoV-2 utilizing QIAamp RNA Viral Mini Kit (cat. no. 52904/ 52906, Qiagen) was confirmed to be 0.8 copies/µL (800 copies/mL). For the HighPrep Viral DNA/RNA Kit (MagBio, CAT#HPV-DR96) the LoD was confirmed to be 1.0 copies/µL (1,000 copies/mL). For the Viral DNA/RNA Kit (CW Bio, CAT#CW3126M) the LoD was confirmed to be 2.0 copies/µL (2,000 copies/mL). For the sbeadex Viral RNA Purification Kit was run on the oKtopure (Biosearch Technologies, CAT#NAP-40-026-04) the LoD was confirmed to be 6.0 copies/µL (6,000 copies/mL).

11.3 Inclusivity (Analytical Sensitivity)

11.3.1 *In Silico* Inclusivity

An alignment was performed with the oligonucleotide CoPrimer sequences of the COVID-19 CoPrimers with all publicly available nucleic acid sequences for SARS-CoV-2 in GenBank, as well as the GISAID database to demonstrate the predicted inclusivity of the Logix Smart SARS-CoV-2 (genes RdRp/E) Test.

Co-Diagnostics has been performing consistent reviews of the sequence alignment to monitor the sequence conservation by analyzing phylogenic mutation genomic data pulled by NextStrain from the GISAID database. The first alignment was performed on 4-Feb-2020 with posterior queries performed on March, April, May, and June, July, August, and September. Partial and cumulative results are displayed. Sequences were obtained from https://github.com/nextstrain/ncov/blob/master/data/metadata.tsv

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Table 13

In Silico Analysis History

Date of CoDx's Analysis for RdRp Marker	SARS-CoV-2 samples analyzed number of sequences in analyzed subsample	Sequences in the pool with 100% homology	Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)	Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)	Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)
27-Jan-20	14	14 (100%)	0 (0%)	0 (0%)	0 (0%)
4-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)
17-Mar-20	571	570 (99.8%)	1 (0.2%)	0 (0%)	0 (0%)
6-Apr-20	3639	3634 (99.86%)	5 (0.14%)	0 (0%)	0 (0%)
4-May-20	4468	4459 (99.80%)	9 (0.2%)	0 (0%)	0 (0%)
3-Jun-20	4558	4537 (99.54%)	21 (0.46%)	0 (0%)	0 (0%)
6-Jul-20	11361	11328 (99.71%)	33 (0.29%)	0 (0%)	0 (0%)
10-Aug-20	22054	22012 (99.81%)	42 (0.19%)	0 (0%)	0 (0%)
9-Sep-20	4417	4394 (99.48%)	23 (0.52%)	0 (0%)	0 (0%)
12-Oct-20	5139	5114 (99.51%)	25 (0.49%)	0 (0%)	0 (0%)
27-Jan-20	14	14 (100%)	0 (0%)	0 (0%)	0 (0%)
4-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)
9-Sep-20	4417	4400 (99.62%)	14 (0.32%)	2 (0.05%)	1 (0.2%)
12-Oct-20	5139	5126 (99.96%)	11 (0.21%)	0 (0%)	2 (0.04%)

Each marker in Logix Smart SARS-CoV-2 is expected to detect strains with a single mismatch without difficulty. At 2 mismatches, each marker in Logix Smart SARS-CoV-2 is expected to detect with significant Cq delay. Events of 3+ mismatches are expected to lead to no detection by that marker. To maintain 99%+ expected sensitivity for both markers, 99%+ of the sampled sequences should maintain less than three mismatches on either marker. To maintain 99%+ expected sensitivity for either marker, 99%+ of the sampled sequences should main should maintain <3 mismatches on both markers.

The alignment data and posterior updated analyses have shown less than three mismatches for both the forward and reverse CoPrimers on 100% of sequences for the RdRp marker and 99.96% of sequences for the E-Gene marker in the NextStrain Global Subsampling of the GISAID database. Therefore, there is a ~0.04% prediction of false-negative results for the E-Gene marker alone and no prediction of false-negative results for both markers together based upon the available data.

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11.3.2 Wet-Test Inclusivity

Inclusivity wet testing was performed to confirm that the Logix Smart SARS-CoV-2 can detect multiple SARS-CoV-2 strains/isolates. Testing was performed by spiking negative extracted saliva or sputum matrix at 9x, 3x, and 1x LoD, run in quadruplicate. See Table 14 for the testing results.

Table 14

Logix Smart SARS-CoV-2 Inclusivity Testing Results

SARS-CoV-2 Strain	Target	Average Ct ± S.D.	
USA-CA3/2020	RdRp	**9x LoD**	31.97 ± 0.32
		3x LoD	33.26 ± 0.29
		1x LoD	33.86 ± 0.33
	E-gene	**9x LoD**	31.27 ± 0.11
		3x LoD	33.09 ± 0.35
		1x LoD	33.45 ± 0.27
USA-IL1/2020	RdRp	**9x LoD**	30.89 ± 0.23
		3x LoD	32.43 ± 0.19
		1x LoD	34.28 ± 0.51
	E-gene	**9x LoD**	30.11 ± 0.17
		3x LoD	31.74 ± 0.36
		1x LoD	33.90 ± 0.52
Italy-INMI1/2020	RdRp	**9x LoD**	30.27 ± 0.14
		3x LoD	31.76 ± 0.23
		1x LoD	33.61 ± 0.42
	E-gene	**9x LoD**	30.27 ± 0.07
		3x LoD	31.90 ± 0.12
		1x LoD	33.76 ± 0.18
Germany/BavPat1/2020	RdRp	**9x LoD**	31.47 ± 0.07
		3x LoD	33.45 ± 0.37
		1x LoD	34.80 ± 0.21
	E-gene	**9x LoD**	32.40 ± 0.19
		3x LoD	34.42 ± 0.63
		1x LoD	35.47 ± 0.78
USA-AZ1/2020	RdRp	**9x LoD**	30.35 ± 0.28
		3x LoD	34.30 ± 0.71
		1x LoD	35.88 ± 1.29
	E-gene	**9x LoD**	30.06 ± 0.12
		3x LoD	34.10 ± 0.72
		1x LoD	34.97 ± 0.18
Hong Kong/VM20001061/2020	RdRp	**9x LoD**	30.08 ± 0.22
		3x LoD	31.62 ± 0.25
		1x LoD	33.10 ± 0.24
	E-gene	**9x LoD**	29.96 ± 0.16
		3x LoD	31.53 ± 0.43
		1x LoD	33.24 ± 0.04

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11.4 Cross-Reactivity (Analytical Specificity) by an *In Silico* Analysis:

In Silico Analysis BLASTn analysis queries of the SARS-CoV-2 CoPrimers 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); 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. 7) BLASTn was run individually for every organism requested by the FDA EUA pre-submission template guidelines for in silico analysis of microorganism of relevance. Table XXX displays the list of microorganisms requested for the FDA EUA.

It is expected that the E gene marker will efficiently amplify many strains of both Bat SARS-like coronavirus as well as Human SARS coronavirus. It is not expected that the E gene marker will cross-amplify with any other coronaviruses, human microflora, or any other organisms that have been sequenced in the NCBI database.

CoPrimers have a slightly different cross-reactivity risk profile than traditional primers. Due to the low Tm's of the Priming and Capture sequences, CoPrimers are more susceptible to mismatches. Our internal experiments show that a single mismatch on either forward or reverse causes a noticeable delay in amplification, with more mismatches causing significant suppression of signal. 3+ mismatches on the forward and reverse combined are expected to result in no detectable amplification.

The results suggest that the Logix Smart SARS-CoV-2 (genes RdRp/E) kit does not cross-react to any of the non-target organisms that were tested in the wet test or in silico analysis. The negative samples did not show any amplification, therefore, no false positives occurred due to cross-reactivity. Positive samples in the presence of non-target organism genetic material in most cases did not reduce the ability of the Logix Smart SARS-CoV-2 (genes RdRp/E) kit to produce positive results. See Table 15.

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Table 15

Microorganism Included in The Cross-Reactivity In Silico Assessment

High priority pathogens from the same genetic family	High priority organisms likely in the circulating area	Other microorganisms of importance
Human coronavirus 229E	Adenovirus	Influenza C
Human coronavirus OC43	Human Metapneumovirus (hMPV)	Parechovirus
Human coronavirus HKU1	Parainfluenza virus 1-4	Corynebacterium diphtheriae
Human coronavirus NL63	Influenza A & B	Legionella non-pneumophila
SARS-coronavirus	Enterovirus	Bacillus anthracis (Anthrax)
MERS-coronavirus	Respiratory syncytial virus	Moraxella catarrhalis
	Rhinovirus	Neisseria elongata
	Chlamydia pneumoniae	Neisseria meningitides
	Haemophilus Influenza	Leptospirosis
	Legionella pneumophila	Chlamydia psittaci
	Mycobacterium tuberculosis	Coxiella burnetii (Q-Fever)
	Streptococcus pneumoniae	Staphylococcus aureus
	Streptococcus pyogenes	
	Bordetella pertussis	
	Mycoplasma pneumoniae	
	Pneumocystis jirovecii (PJP)	
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract	
	Candida albicans	
	Pseudomonas aeruginosa	
	Staphylococcus epidermidis	
	Staphylococcus salivarius	

11.5 Wet-Test Exclusivity

Exclusivity wet testing was performed to confirm that the **Logix Smart SARS-CoV-2 (genes RdRp/E) kit** does not cross react with non-target organisms. The test was performed by spiking negative sputum, with non-target organisms, or the non-target organism's extracted genome. The materials that were already extracted were spiked post extraction. Non-target organisms were spiked in at a final concentration

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of 1e4 copies/rxn (1e3 copies/µL [1e6 copies/mL]) and run in duplicate. Additionally, to verify that the presence of non-target genomic DNA/RNA does not affect the ability to detect SARS-CoV-2, non-target organisms were spiked in at a final concentration of 1e4 copies/rxn (1e3 copies/µL [1e6 copies/mL]) and the AMPLIRUN® Coronavirus SARS-CoV-2 RNA Control was spiked in at 3x LoD, and run in triplicate.

The data generated from the specificity-exclusivity runs are summarized in Table 16. Based on the results, the presence of the non-target organism's genomic material did not significantly affect the amplification of either the *RdRp* target or the *E* gene target ≥ 2 Cq. Additionally, there was no amplification in the reactions that included only the non-target organism, with the exception of SARS-CoV-1 (2003) which was expected to amplify based on the *in silico* analysis.

Table 16

Logix Smart SARS-CoV-2 (genes RdRp/E) Exclusivity Testing

Sample	Target	Average Ct ± S.D.
Human coronavirus OC43	RdRp	34.91 ± 0.27
	E-gene	33.55 ± 0.20
Human coronavirus HKU1	RdRp	34.48 ± 0.05
	E-gene	33.76 ± 0.35
Human coronavirus NL63	RdRp	35.12 ± 1.10
	E-gene	33.99 ± 0.54
SARS-coronavirus	RdRp	35.08 ± 0.46
	E-gene	23.22 ± 0.13
MERS-coronavirus	RdRp	35.12 ± 0.88
	E-gene	34.94 ± 1.86
Human Metapneumovirus (hMPV)	RdRp	35.06 ± 0.40
	E-gene	34.04 ± 0.54
Parainfluenza virus 3	RdRp	34.88 ± 0.30
	E-gene	33.51 ± 0.03
Influenza A	RdRp	34.91 ± 0.23
	E-gene	33.38 ± 0.20
Influenza B	RdRp	34.99 ± 1.04
	E-gene	33.65 ± 0.45
Enterovirus (e.g., EV68)	RdRp	35.21 ± 0.19
	E-gene	33.20 ± 0.01
Respiratory syncytial virus	RdRp	36.20 ± 1.44
	E-gene	33.94 ± 0.28

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Sample	Target	Average Ct ± S.D.
Rhinovirus	RdRp	35.56 ± 0.38
	E-gene	35.29 ± 0.38
Respiratory Viral Panel	RdRp	35.21 ± 0.06
	E-gene	35.22 ± 0.62
Chlamydia pneumoniae	RdRp	34.52 ± 0.25
	E-gene	33.26 ± 0.79
Haemophilus influenzae	RdRp	35.78 ± 1.54
	E-gene	34.08 ± 0.81
Legionella pneumophila	RdRp	35.09 ± 0.73
	E-gene	34.24 ± 1.00
Mycobacterium tuberculosis	RdRp	34.98 ± 0.89
	E-gene	35.05 ± 0.49
Streptococcus pneumoniae	RdRp	36.46 ± 0.64
	E-gene	35.64 ± 1.29
Streptococcus pyogenes	RdRp	35.57 ± 0.39
	E-gene	35.45 ± 0.49
Bordetella pertussis	RdRp	35.32 ± 0.51
	E-gene	35.65 ± 0.43
Mycoplasma pneumoniae	RdRp	35.41 ± 0.54
	E-gene	35.73 ± 0.58
Pneumocystis jirovecii (PJP)	RdRp	35.69 ± 0.41
	E-gene	35.65 ± 0.22
Candida albicans	RdRp	35.28 ± 0.86
	E-gene	35.46 ± 0.59
Pseudomonas aeruginosa	RdRp	35.12 ± 0.71
	E-gene	35.65 ± 0.18
Staphylococcus epidermidis	RdRp	36.61 ± 1.60
	E-gene	35.51 ± 0.95
Streptococcus salivarius	RdRp	36.80 ± 0.89
	E-gene	35.03 ± 0.36
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract	RdRp	34.52 ± 0.39
	E-gene	36.16 ± 0.74

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11.6 Diagnostic Accuracy

Data from the runs of LoD and precision experiments were aggregated and analyzed. The totals of 2364 readings with concentrations above the limit of detection were collected for the true negatives (TN), false positives (FP), true positives (TP), and false negatives (FN). Additionally, the values for sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), and Michaels Correlation Coefficient (MCC) have been calculated. See Table 17 for diagnostic accuracy for Logix Smart SARS-CoV-2 (genes RdRp/E).

Table 17

Diagnostic Accuracy for Logix Smart SARS-CoV-2 (genes RdRp/E)

	SARS-CoV-2
True Negatives (TN)	906
False Positives (FP)	10
True Positives (TP)	1440
False Negatives (FN)	8
Sensitivity	99.448%
Specificity	98.908%
Accuracy	0.992
PPV	0.993
NPV	0.991
MCC	0.984

11.7 Performance Summary

See Table 18 for the performance summary for Logix Smart SARS-CoV-2 (genes RdRp/E).

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Table 18

Performance Summary for Logix Smart SARS-CoV-2 (genes RdRp/E)

Application	Qualitative Multiplex PCR test for the detection of SARS-CoV-2	
Limit of Detection (copies/mL)	QIAamp Viral RNA Mini Kit (Qiagen)	800 copies/mL
	HighPrep Viral DNA/RNA Kit (MagBio)	1,000 copies/mL
	Viral DNA/RNA Kit (CW Bio)	2,000 copies/mL
	sbeadex Viral RNA Purification Kit automated with oKtopure High Throughput system (both LGC Biosearch Technologies)	6,000 copies/mL
Sensitivity*	99.448%	
Specificity*	98.908%	
Sample type	Lower respiratory samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva	
Time to detection	Approximately 90 minutes, depending on the instrument used	
Thermal cycler compatibility	•CoDx Box (Co-Diagnostics, Inc.) •Mic cycler (BMS, Biomolecular Systems) •QuantStudio 5 (Thermo Fisher Scientific) •CFX96 (Bio-Rad) The test should work with most qPCR systems with the following channel compatibilities: FAM CF560 (VIC) CF610 (ROX)	
Extraction kit compatibility	QIAamp® Viral RNA Mini Kit (Qiagen, CAT#52906) HighPrep Viral DNA/RNA (MagBio Genomics, CAT#HPV-DR96) Viral DNA/RNA Kit (CW Bio, CAT#CW3126M) sbeadex Viral RNA Purification Kit (LGC Biosearch Technologies, CAT#NAP-40-026-04).	

*Results obtained from observational study from 2364 runs of contrived samples.

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12 MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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13 REFERENCES

CDC. (2009). *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.* Retrieved from CDC Laboratories: https://www.cdc.gov/labs/BMBL.html

CDC. (2020, Feb 7). *Coronavirus Disease 2019 (COVID-19): Information for Laboratories.* Retrieved from Centers for Disease Control and Prevention: https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf

CDC. (2020, Feb 16). *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).* Retrieved September 15, 2018, from World Health Organization: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Flab-biosafety-guidelines.html

Centers for Disease Control and Prevention. (2017, Oct 27). *CDC Laboratory Training: Good Laboratory Practices for Molecular Genetics Testing*. Retrieved Mar 5, 2019, from CDC: https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html

Poritz, M., & Ririe, K. (2014, Mar). Getting things backwards to prevent primer dimers. *Journal of Molecular Diagnosis*, 159-62. doi:10.1016/j.jmoldx.2014.01.001

Satterfield, B. (2014, Mar). Cooperative primers: 2.5 million-fold improvement in the reduction of nonspecific amplification. *Journal of Molecular Diagnosis*, 163-73. doi:10.1016/j.jmoldx.2013.10.004

Viana, R. V., & Wallis, C. L. (2011). Good Clinical Laboratory Practices (GLCP) for Molecular Based Tests Used in Diagnostic Laboratories. In D. I. Akyar, *Wide Spectra of Quality Control* (pp. 29-52). InTech. Retrieved from http://www.intechopen.com/books/wide-spectra-of-quality-control/goodclinical-laboratory-practice-gclp-for-molecular-based-tests-used-in-diagnostic-laboratories

WHO. (2004). *Laboratory Biosafety Manual.* Retrieved from Emergencies preparedness, response: https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

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14 LEGEND OF PACKAGE SYMBOLS

See Table 19 for a legend of the package symbols

Table 19

Legend of Package Symbols

Icon	Description
IVD	*In vitro* diagnostic medical device
REF	Catalog number
LOT	Batch code
CAP	Cap color
COMP	Component
CONT	Content/Volume
NUM	Number
	Use-by-date
Σ	Contains sufficient for 100, 250 or 5,000 reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
NON STERILE	Non-sterile product - Do not sterilize.
	Manufacturer
EC REP	Authorized representative in the European Community
CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC

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