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Instructions for Use
Co-Dx™ Logix Smart® SARS-CoV-2 (genes RdRp/E) Kit

For in vitro diagnostic use

REF **COVID-K-002-0100**
COVID-K-002-0250

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

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

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1 INTENDED USE

The **Co-Dx™ Logix Smart® SARS-CoV-2 (genes RdRp/E)** kit is a real-time, polymerase chain reaction (rRT-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 swabs, oropharyngeal swabs), and saliva from individuals suspected of having contracted COVID-19.

Results are used for the identification of SARS-CoV-2 ribonucleic acid (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 swabs, 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 **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit is intended for use by qualified and trained clinical laboratory personnel specifically who are instructed and trained in the techniques of rRT-PCR and *in vitro* diagnostic procedures.

2 PRODUCT DESCRIPTION AND TEST PRINCIPLE

The **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit is a real-time, reverse transcription polymerase chain reaction (rRT-PCR) multiplex test utilizing the Company's patented Co-Primer® technology (Satterfield, 2014) (Poritz & Ririe, 2014). The two sets of SARS-CoV-2 Co-Primer (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 having contracted COVID-19.

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

- Ready-to-use Master Mix (MM), including Ribonuclease P (RNaseP) internal positive control (IPC) to verify sample quality

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- A Positive Control (PC), to verify the performance of the MM
- Negative Control (NC) to verify the MM is free of contamination

2.1 Principles of Operation

The following steps are recommended during operation:

2.1.1 Select a sample type.

2.1.2 Have a trained healthcare provider collect the sample.

2.1.3 Identify the sample by following the laboratory quality system and current regulation.

2.1.4 Store the sample properly until testing occurs (either in the same facility or during shipping to the assigned laboratory).

The **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit assay is a multiplex, single-step, rRT-PCR test that can be broken down into the following three stages:

- Sample preparation
- Reverse transcription
- PCR with real-time monitoring

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

The sample preparation for PCR requires the samples are 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, oropharyngeal swabs), and saliva from patients who are suspected of COVID-19.

The purified nucleic acid is then plated with the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit MM (5 µL of each). The MM 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.

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Place the plated reactions in the thermocycler and perform each of the cycling conditions listed below in the following order:

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

The first step (15 min at 45°C) is the reverse-transcription step, where the cDNA is created from the RNA template.

The second step (2 min at 95°C) is performed to inactivate the reverse transcriptase and acts as the initial denaturation step for PCR, which is followed by thermocycling for the PCR.

During the PCR, the labeled forward Co-Primers act as both the forward primer and probe. During the annealing/extension phase of the PCR, the 5' nuclease activity of Taq polymerase degrades the Co-Primer's portion that annealed to the targeted sequence, causing spatial separation between the fluorophore and the quencher, and generating a fluorescent signal.

With each cycle, additional fluorophore molecules 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. See Table 1 for the components included in this kit.

Table 1

Kit Components

Cap Color	Component	Symbol	Description	Individual Catalog Number
Brown	**Co-Dx Logix Smart SARS-CoV-2** Master Mix	MM	Proprietary blend of SARS-CoV-2 Co-Primer and PCR reagents	TUBE-CV2-0001 (1x500 µL [100 reactions]) TUBE-CV2-0010 (1x1250 µL [250 reactions])
Clear	**Co-Dx Logix Smart SARS-CoV-2** Negative Control	NC	Nuclease-Free Water	TUBE-CV2-0002 (1x500 µL [100 reactions]) TUBE-CV2-0020 (1x1250 µL [250 reactions])
Red	**Co-Dx Logix Smart SARS-CoV-2** Positive Control	PC	Proprietary blend of SARS-CoV-2 synthetic templates	TUBE-CV2-0003 (1x500 µL [100 reactions]) TUBE-CV2-0030 (1x1250 µL [250 reactions])

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The product code is COVID-K-002-0100 for the 100 reaction size kit and COVID-K-002-0250 for the 250 reaction size kit. Contact the Co-Diagnostics' Sales department at (801) 438-1036 ext. 1 or go to www.co-dx.com/contact/ to order.

3 REAGENT STORAGE AND HANDLING

3.1 Steps

Perform the following steps when storing and handling reagents:

3.1.1 Ensure the kit components arrive frozen.

> **Note:** The **Co-Dx 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.

3.1.2 Immediately store all components at a temperature between -40°C and -16°C to prevent degradation of reagents.

3.1.3 Always work with each **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit component on ice. (Make aliquots, if necessary, to avoid multiple freeze/thaw cycles.)

3.1.4 Ensure that the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit always remains frozen at a temperature between -40°C and -16°C. Keep a back-up generator for your freezer as well as a temperature data log if you work in an area prone to power outages.

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

4 MATERIAL REQUIRED BUT NOT INCLUDED WITH THE TEST

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

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Table 2

Extraction and Automation Systems Validated with the Test

Extraction Reagent		Automation Platform (if applicable)	Manufacturer	Sample Input Volume/Sample Elution Volume
Name	Catalog 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
Co-Dx 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

4.1 Consumables Required but Not Provided

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

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

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4.2 Equipment Required but Not Provided

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

- Several micropipettes capable of pipetting volumes from 5 µL to 1000 µL
- A cold block or ice
- A vortex and a centrifuge
- Class II biosafety cabinet, ideally used in a BSL-2 containment facility, for the extraction
- PCR workstation, for MM 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 are 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 **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit components and the qPCR technology, take care while handling samples and materials and while performing the assay to keep reagents and amplification mixtures free of contamination.

5.1 Steps to Follow

Ensure the following steps are taken:

5.1.1 Use sterile pipette tips with filters.

5.1.2 Use standard precautions when handling any patient samples, as they may contain infectious agents.

5.1.3 Store and extract positive materials (specimens, PCs, and amplicons) separately from other reagents.

5.1.4 Use the NC provided with this kit.

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5.1.5 Consult appropriate Safety Data Sheets (SDS) for safe handling of the kit. The SDS for the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit should be provided with the shipment. If it is not provided with the shipment, retrieve the SDS from Co-Diagnostics website at the link: Product Information - Co-Dx.

5.1.6 Use Good Laboratory Practices for Molecular Biology which requires a unidirectional workflow and the separation of negative and positive materials to prevent contamination.

5.1.7 Always use the most recent version of this document as more information is added with future studies. The most recent version of this document can be downloaded for free at Product Information - Co-Dx.

6 SAMPLE COLLECTION, HANDLING, SHIPPING, AND STORAGE

The sample collection, handling, shipping, and storage play an essential part in the performance of nucleic acid assays. If the laboratory does not have internal procedures for patient specimen selection, collection, storage, and handling, 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 Center for Disease Control and Prevention (CDC) and World Health Organization (WHO) websites at 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 Sample Collection – Lower Respiratory Tract Fluids

Following are instructions for various methods of gathering lower respiratory tract fluids:

6.1.1 Bronchoalveolar Lavage, or Tracheal Aspirate

6.1.1.1 Collect 2-3 mL into a sterile, leak-proof, screwcap, sputum-collection cup, or sterile dry container.

6.1.1.2 Refrigerate the specimen at a temperature between 2°C and 8°C and ship overnight to the testing laboratory on an ice pack.

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6.1.2 Sputum

6.1.2.1 Instruct the patient to rinse their mouth with water.

6.1.2.2 Instruct the patient to expectorate their deep-cough sputum directly into a sterile, leak-proof, screwcap, sputum collection cup or sterile dry container.

6.1.2.3 Refrigerate the specimen at a temperature between 2°C and 8°C and ship overnight to the testing laboratory on an ice pack.

6.2 Sample Collection – Upper Respiratory Tract Fluids

Following are instructions for various methods of gathering upper respiratory tract fluids:

6.2.1 Nasopharyngeal (NP) Swab and Oropharyngeal (OP) Swab

Important: When collecting NP/OP swab samples, use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, because they may contain substances that inactivate some viruses and inhibit PCR testing.

6.2.1.1 Place swabs immediately into sterile tubes containing viral transport media. NP and OP specimens should be kept in separate vials. Refrigerate the specimen at a temperature between 2°C and 8°C and ship overnight to the testing laboratory on an ice pack.

Note: To use an NP swab, insert the swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both NP areas with the same swab.

6.2.2 Oropharyngeal Swab (OP)

6.2.2.1 To obtain an OP sample, swab the posterior pharynx, avoiding the tongue.

6.2.3 Nasopharyngeal Wash/Aspirate or Nasal Aspirate

6.2.3.1 Collect 2-3 mL of sample into a sterile, leak-proof, screw-cap collection cup or sterile dry container.

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6.2.3.2 Refrigerate specimen at a temperature between 2°C and 8°C and ship overnight to the testing laboratory on an ice pack or collect the sample in a vial with virus transport media, which does not demand refrigeration or cold-chain transport but may require validation by the laboratory.

6.3 Sample Collection - Saliva

Collect saliva samples by following these steps:

6.3.1 Collect 2-3 mL of saliva into a sterile, leak-proof, screw-cap container, or collect saliva up to the fill line for screw-cap saliva collection kits.

6.3.2 Refrigerate the specimen at a temperature between 2°C and 8°C and ship overnight to the testing laboratory on an ice pack.

Note: Follow the manufacturer's instructions when collecting, storing, and shipping saliva collection kit contents.

6.3.3 Adhere to the following procedures when handling samples:

6.3.4 Wear appropriate personal protective equipment, including disposable gloves, laboratory coat/gown, and eye protection when handling potentially infectious specimens.

6.3.5 Collect specimens, from patients suspected of being infected with or confirmed to be infected with COVID-19, under a certified Class II Biosafety cabinet in a Biosafety Level 2 containment facility. The *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (CDC, 2009) or the *WHO Laboratory Biosafety Manual* (WHO, 2004) provides more information.

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

6.4 Sample Shipping

6.4.1 Adhere to the following guidelines when shipping samples by air:

6.4.1.1 Ship specimens that you know (or suspect) contain SARS-CoV-2, and require shipment by air, on dry ice as Biological Substance Category B, UN3373.

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6.4.1.2 Follow international regulations, as described in the WHO Guidance on Regulations for the Transport of Infectious Substances 2015-2016 (CDC, 2020).

6.4.2 Adhere to the following guidelines when shipping samples by ground:

6.4.2.1 Ship specimens frozen overnight with enough ice to keep specimens frozen throughout transit.

6.4.2.2 After the collection of the sample and the transfer to the clinical lab, the sample will receive an entry into the laboratory system.

6.5 Sample Storage

Adhere to the following when storing specimen samples:

6.5.1 Keep all specimen types at -16°C for up to 7 days.

6.5.2 If storing for longer than 7 days, keep specimens frozen at a temperature between -40 °C and -16°C.

6.5.3 Avoid repeated freezing and thawing of a specimen.

6.5.4 If keeping a specimen for retesting, aliquot it in different tubes to avoid freezing and thawing cycles.

6.5.5 Monitor and record the temperature in the storage areas regularly to identify potential fluctuations.

Note: Domestic refrigerators/freezers with wide temperature fluctuations are not suitable for the storage of frozen specimens (CDC, 2020).

7 PROCEDURE

The WHO recommends recording the full name, date of birth, contact information, and the time and date of collection of the patient sample. Additionally, collect the following information as needed:

- Symptoms
- Date of onset
- Duration of symptoms
- Contact with known COVID-19 cases (e.g., sister, father)
- Comprehensive travel history (i.e., dates, place, duration of visit)

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7.1 Sample Preparation

The quality of the RNA from the extraction of the sample is essential to the performance of **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit.

7.1.1 Extraction of RNA with QIAamp® Viral RNA Mini Kit, cat. no. 52904/52906, Qiagen

7.1.1.1 Perform the extraction by following the manufacturer's instructions, using 140 µL of the sample and an elution using 60 µL of buffer AVE.

7.1.1.2 For additional sensitivity, load up to 200 µL of patient sample and increase the volume of Buffer AVL from 560 µL to 800 µL.

7.1.1.3 To ensure the removal of residual wash buffer from the sample prior to elution, perform an additional centrifugation step (see extraction procedure) using a new collection tube.

7.1.1.4 Due to the mucoid, mucopurulent, and viscous nature of sputum specimen, pre-process the sample before extraction.

Note: 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).

Important: Only perform processing in conjunction with the QIAamp Viral RNA Mini kit.

7.1.1.5 Incubate the sample and mix occasionally at room temperature until the sample is liquified. (This step can take up to 30 minutes.)

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7.1.1.6 Use the liquified sample for downstream nucleic acid extraction by following the extraction system manufacturer's guidelines.

7.1.1.7 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 Do the following when extracting sputum or saliva samples:

7.1.3.1 Collect sputum samples directly with a 50 mL conical tube, dilute 1:8 with 10% TE buffer, and vortex.

7.1.3.2 Collect the 2 mL saliva samples with the spectrum solution DNA collection device.

7.1.3.3 Dilute saliva samples with 1.5 mL of CV3 Spectrum Chemistry transport media, further dilute with 10% TE buffer, and vortex for a final 1:3 dilution.

7.1.3.4 Prepare the bead/binding mixture.

7.1.3.5 For each plate being processed by the oKtopure, combine 46.1 mL of binding buffer SB and 2.9 mL of sbeadex particles. For each additional plate, multiply appropriately.

7.1.4 Perform the following protocol for a single, 96-well plate:

7.1.4.1 In a fresh oKtopure 96-well sample plate, add 200 µL of sample into each well. If there are remaining wells of the plate that are not being used for a sample, add 200 µL of NC into the wells.

7.1.4.2 Once all wells are filled with either sample or the NC, securely place the 96 well plate inside the oKtopure.

7.1.4.3 Note: Reference the oKtopure software for the proper placement/location of reservoirs and 96 well plates for each in the following steps below.

7.1.4.4 Measure 30 mL of lysis buffer into a clean reservoir, and securely place the lysis buffer into the oKtopure.

7.1.4.5 Add 200 µL of lysis buffer to each well by the oKtopure.

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7.1.4.6 Measure 48 mL of bead/binding mixture into a clean reservoir.

7.1.4.7 Add 340 µL of bead/binding mixture to each well by the oKtopure.

7.1.4.8 Move the 96 well sample plate to the appropriate position and remove the bead/binding mixture in the reservoir.

7.1.4.9 Collect the following:

- Four clean reservoirs
- One lysis waste plate (96 square welled plate)
- Ninety-six well elution plate (destination plate)

7.1.4.10 Place the lysis waste plate and the 96 well elution plate in the appropriate position.

7.1.4.11 Add the following volumes of buffer into each reservoir:

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

7.1.4.12 Verify the template run file (200 µL sbeadex) and start the extraction.

7.1.4.13 Allow the oKtopure to fill following volumes of buffer into each well:

- BN1 to 300 µL
- TN1 to 240 µL
- TN2 to 370 µL
- Elution Buffer to 60 µL

7.1.4.14 Either keep the samples on ice or use immediately to run the PCR. Store extra extracted material at -80°C.

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7.1.5 Manual Extraction of RNA Using the Viral DNA/RNA kit, Cat No. CW3123S/ CW3123M/ CWY070, CWBio

7.1.5.1 Follow the manufacturer's instructions for a 200 µL of sample, and a 60 µL elution volume.

7.1.6 Manual extraction of RNA Using the HighPrep Viral DNA/RNA Kit, Cat No. HPV-DR96, MagBio

7.1.6.1 Follow the manufacturer's instructions for a 200 µL of sample, and a 60 µL elution volume.

Warning!

The 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 rRT-PCR.

7.2 Set up the Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E) Reagent

Perform these steps to set up the reagent:

7.2.1 Clean all working surfaces with a fresh 10% bleach solution followed by a molecular-grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.

7.2.2 Vortex all **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** MM, PC, NC, and all sample tubes for 3 seconds.

7.2.3 Briefly spin the MM, PC, NC down before using to ensure reagents are properly mixed and to ensure removal of any condensation or residue from the lids.

7.2.4 Thaw all reagents and samples on ice, or a cold block, before starting the setup.

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7.3 Set Up the Reaction

Perform the steps below to set up the reaction.

7.3.1 Collect enough reaction wells for each of the following:

- One for each NC,
- One for each sample you want to test, and
- One (or more) for each PC

Note: The example below displays the minimum number of wells needed for 5 samples.

PC	1
NC	1
Samples	5
Total wells required	**7**

7.3.2 Pipette 5 µL of MM into each well collected.

7.3.3 Pipette 5 µL of the NC into the appropriate wells (in addition to the 5 µL of MM already in the well).

Note: Ensure that at least one NC is included in each run and that enough space remains for at least one PC.

Important:

- Pipette on ice, if possible.
- Perform PC pipetting and sample setup in a separate area, or at a separate time from the MM and NC.
- Change pipette tips between samples and change pipette tips after pipetting each component.
- Pipette the PC last, if possible, to avoid contamination events.

7.3.4 Pipette 5 µL of sample or PC into the appropriate well.

7.3.5 Seal the reaction plate with an optical adhesive film or seal each reaction tube with its appropriate lid.

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

7.4 Thermocycler Setup

7.4.1 qPCR Instrument Setup for the Co-Dx Box

7.4.1.1 Contact the Laboratory at www.co-dx.com/contact/ to download the template file for use with the Co-Dx Box which comes pre-programmed with the PCR instrument setup described in the qPCR Instrument Setup section.

7.4.2 qPCR Instrument Setup

7.4.2.1 Define the settings as displayed in Table 4.

Table 4

Settings to Define

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

7.4.2.2 Program PCR instrument with the cycling conditions displayed 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

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

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Table 6

Fluorescence Detector Definitions

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

7.4.2.4 When the run finishes, save the run file.

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 **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit 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

8.1.1 Set the following analysis parameters on the Co-Dx Box or Mic qPCR Cycler, and after every run, verify 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):

8.1.1.1 Click to activate the **Auto Set Threshold** checkbox.

8.1.1.2 Set the **Method** to *Dynamic*.

8.1.1.3 Set the **Threshold Level** to *0.100*.

8.1.1.4 Set the **Threshold Start** to *1.00*.

8.1.1.5 Set the **Ignore Cycles Before** to *5*.

8.1.1.6 Set **Exclusion** to *Extensive*.

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8.1.1.7 Set the **Fluorescence Cutoff Level** to *5.0%.*

8.1.1.8 Set the **Initial Y-Axis Scale** to *Linear*.

8.1.1.9 Click to activate the **Auto Generate Analysis** checkbox.

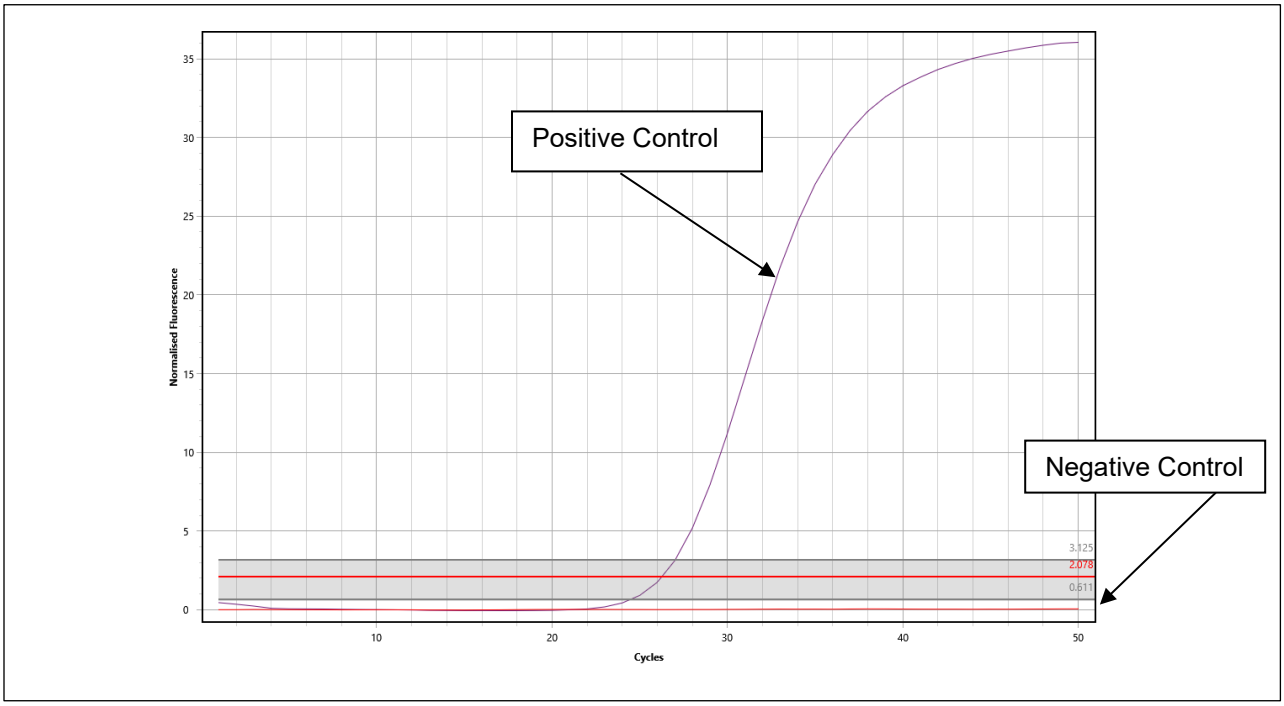
8.1.2 For other thermocyclers, follow the manufacturer's instructions for setting an appropriate threshold.

8.2 Positive Control

Highlight the PC reaction well. Each PC 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 PC for RNaseP IPC in the CF610 channel. A positive amplification curve looks like the purple curve in Figure 1 and should have a Cq value below 40 cycles.

Figure 1

Positive Control (PC) and Negative Control (NC) Signals for ***Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)***



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8.3 Negative Control (NC)

8.3.1 Highlight the NC.

8.3.2 Ensure the results of the negative control display 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 displayed as the grey band with the red line.

8.4 Validity of the Diagnostic Test Runs

8.4.1 Check to see that both the Positive Control (PC) and the NC have passed.

8.4.2 Ensure the control conditions displayed in Table 7 are 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	RNaseP Internal Positive Control (RNaseP ICP) CF610 channel
SARS-CoV-2 Positive Control (PC)	SARS-CoV-2 *RdRp* (FAM™) SARS-CoV-2 *E* (CF®560) RNaseP IPC (CF®610)	Verifies the performance of the master mix	+	+	+
Negative Control (NC)	Nuclease-Free Water	Verifies the reagents are free of contamination	-	-	-

8.4.3 If controls pass, interpret the sample results.

8.5 Invalid Diagnostic Test Run

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

8.5.2 If the RNaseP IPC fails initiate an investigation to eliminate possible splashing, pipetting error, or any other laboratory error.

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8.6 Interpret the Results

Once the controls have passed, interpret the unknown samples based on these three possible outcomes:

- Positive
- Negative
- Invalid

A positive result shows 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 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. A new run of the same sample or a run of another sample from the same patient within the 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 the troubleshooting section and Table 8.

Table 8

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

	Sample Result			Co-Dx Logix Smart SARS-CoV-2 Positive Control	Co-Dx Logix Smart Negative Control (NC)	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**
			+	-	-	
			+	+	+	

An analyte result is positive (+) if the Cq value is <40 or negative (-) if the Cq value is ≥40.
When possible, 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 **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit, even if the recommended steps for troubleshooting solves the issue. To give feedback, complete the Customer Feedback Form by visiting Feedback - Co-Diagnostics, Inc. (co-dx.com).

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

The 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-minute, 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 **Co-Dx Logix Smart SARS-CoV-2** PC not Amplifying

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

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent)
- Incorrect placing of plates or tubes into the rRT-PCR instrument
- Degrading of **Co-Dx Logix Smart SARS-CoV-2** MM or PC (a

result of reagents being at temperatures above -16°C for an extended period)

- Using expired reagents
- Using the wrong reagents

Without further evidence, the run should be considered invalid, and the user should re-test by re-amplification. If the PC 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 PC happens a third time after re-extraction and re-amplification, open a new **Co-Dx Logix Smart SARS-CoV-2** MM or PC, and retest. If still failing, contact Co-Diagnostics Inc. Technical Support by contacting us at support@co-dx.com.

9.3.2 RNaseP IPC Not Amplifying in Patient Samples

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 were present (such as ethanol and heparin).
- The extraction was performed incorrectly.
- The extraction kit used is not compatible with 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., cerebral spinal fluid, urine, cell lysates) may not contain the human RNaseP gene.

9.3.2.1 If IPC (CF610 channel) displays a negative result while SARS-CoV-2 *RdRp/E* (FAM/CF560) channel(s) shows positive result, initiate an internal investigation.

During the investigation, evaluate for one of the following two possible scenarios:

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- 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.

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

9.3.2.3 If the cause for an error is unclear, contact Co-Diagnostics Inc. Technical Support by contacting us at support@co-dx.com or filling out our feedback form at Customer Feedback - Co-Dx (codiagnostics.com).

9.3.3 Negative Control (NC) Displays Amplification

Amplification of SARS-CoV-2 *RdRp/E* in the NC indicates contamination of one or more of the reagents, incorrect placement of plate or tube into the rRT-PCR instrument, or pipetting errors.

9.3.3.1 If this occurs, interpret the results as invalid and perform re-testing by re-amplification.

9.3.3.2 If the NC fails again, investigate to identify potential causes for error, and depending on the investigation results and risks identified in the process, re-extract the patient samples.

9.3.3.3 If failure of the NC, after re-extraction and re-amplification, occurs a third time, open a new NC and retest.

9.3.3.4 If it still fails, interpret the run as invalid and contact Co-Diagnostics Inc. Technical Support at support@co-dx.com.

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10 LIMITATIONS

This kit has been evaluated with the following limitations:

- Strictly comply with the instructions in the document for optimal results. Always use the most recent version of this document which can be downloaded for free at Product Information - Co-Dx.
- Limit the use of this product to personnel trained and instructed in rRT-PCR techniques and IVD procedures.
- Always use good laboratory practices for proper performance of this assay. Upon receipt of reagents, perform a test run to check the performance of the reagents before testing on patient samples.
- Collect, transport, store, and process all specimens appropriately for optimal results.
- Do not use the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit directly on the specimens collected. Perform an appropriate nucleic acid extraction before using this assay.
- Be aware that the presence of PCR inhibitors may cause false negatives or invalid results.
- Be aware that potential mutations of the target regions of the COVID-19 genome covered by this kit may fail to detect the presence of the pathogens.
- As with any diagnostic test, interpret the results of the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit 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, nasopharyngeal fluid, and nasal swab samples acquired from Discovery Life Sciences or donations.

11.1 Precision

The precision study was performed over 10 days with 2 runs 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 were identified in the following way:

- [Normal] = 60 copies/reaction (copies/µL)
- [Low] = 50 copies/reaction (10 copies/µL)

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The average Cqs between days should be less than or equal to 2.0 cycles with variance lower than 5% and p-value lower than 0.05.

Results were found within the acceptance criteria. See Table 9 and Table 10 for more information.

Table 9

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

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

Table 10

Combined Precision Results

Item	Cq Average	SD	Call Rate	CV%	Marker Detection Rate (%)	Kit Detection Rate (%)
RdRp (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%
E gene (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

The 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)
- 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

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kit individually. After the extraction process, the extracts were tested using the **Co-Dx Logix Smart SARS-CoV-2** kit protocol. The LoD was then confirmed by performing 20 replicate extractions at the LoD concentration for each kit. See Table 11.

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%

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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.

Table 12

Confirmation of the LoD

QIAamp Viral RNA Mini Kit (Qiagen)					
Thermocycler	**Concentration (copies/µL)**	**Sample Matrix**	**# Positive**	**Total Samples**	**Detection Rate**
Co-Dx 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**
Co-Dx 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**
Co-Dx 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**
Co-Dx 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 LoD for **Co-Dx 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 Co-Primer sequences of COVID-19 Co-Primers 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 **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** test.

11.3.2 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/.

See Table 13 for information about the In Silico Analysis History.

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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 Co-Dx target (98% homology)	Double nucleotide mutation events: Sequences with 2+ mismatches on Co-Dx target (95% homology)	Multiple nucleotide mutation events: Sequences with 3+ mismatches on Co-Dx 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 **Co-Dx Logix Smart SARS-CoV-2** kit is expected to detect single mismatched strains without difficulty. At 2 mismatches, each marker in **Co-Dx Logix Smart SARS-CoV-2** kit is expected to detect with significant Cq delay. Events of 3 or more 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 maintain less than 3 mismatches on both markers.

The alignment data and posterior updated analyses have shown less than three mismatches for both the forward and reverse Co-Primers 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 about a 0.04% prediction of false-negative results for the

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E-Gene marker alone and no prediction of false-negative results for both markers together, based upon the available data.

11.3.3 Wet-Test Inclusivity

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

Table 14

***Co-Dx 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

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

In Silico Analysis BLASTn analysis queries of the SARS-CoV-2 Co-Primers were performed against public domain nucleotide sequences. The database search parameters were as follows:

- 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 100 Mb.
- 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.
- The search parameters automatically adjust for short input sequences and the expect threshold is 1000.
- The match and mismatch scores are 1 and -3, respectively.
- The penalty to create and extend a gap in an alignment is 5 and 2, respectively.
- 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 15 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.

Co-Primers have a slightly different cross-reactivity risk profile than traditional primers. Due to the low Tms of the Priming and Capture sequences, Co-Primers 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 the signal. Three or more mismatches on the forward and reverse combined are expected to result in no detectable amplification.

The results suggest that the **Co-Dx 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 **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit to produce positive results.

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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	

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11.5 Wet-Test Exclusivity

Exclusivity wet testing was performed to confirm that the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit did 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 of 1e4 copies/rxn (1e3 copies/µL [1e6 copies/mL]) and were run in duplicate.

Additionally, to verify that the presence of non-target genomic DNA/RNA did 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 was 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, except for SARS-CoV-1 (2003), which was expected to amplify based on the *in-silico* analysis.

Table 16

Co-Dx 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

Sample	Target	Average Ct ± S.D.
	E-gene	33.94 ± 0.28
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

11.6 Diagnostic Accuracy

Data from the runs of LoD and precision experiments were aggregated and analyzed. The totals from 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 the diagnostic accuracy of the **Co-Dx Logix Smart SARS-CoV-2 (genes RdRp/E)** kit.

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Table 17

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

Diagnosis	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
Positive Predictive Value (PPV)	0.993
Negative Predictive Value (NPV)	0.991
Michaels Correlation Coefficient (MCC)	0.984

11.7 Performance Summary

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

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Table 18

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

Application	Qualitative Multiplex PCR test for the detection of SARS-CoV-2	
Limit of Detection	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	The following thermo cyclers are compatible with this product: • Co-Dx 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

Manufacturer:
Co-Diagnostics, Inc
2401 S Foothill Dr. Ste D
Salt Lake City, UT 84109
Phone: +1 (801) 438-1036
Email: info@co-dx.com
Website: www.co-dx.com

Authorized Representative:
mdi Europa GmbH
Langenhagener Str. 71
D-30855 Hannover-Langenhagen
Germany
Phone: +49 511 39 08 95 30
Email: info@mdi-europa.com
Website: www.mdi-europa.com

℞ only

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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
	*In vitro* diagnostic medical device
	Catalog number
	Batch code
	Use-by-date
	Contains sufficient for x reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use document
	Non-sterile product - Do not sterilize
	Manufacturer
	Authorized representative in the European Community
	CE-Marking for IVD in compliance to EU Directive 98/79/EC

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