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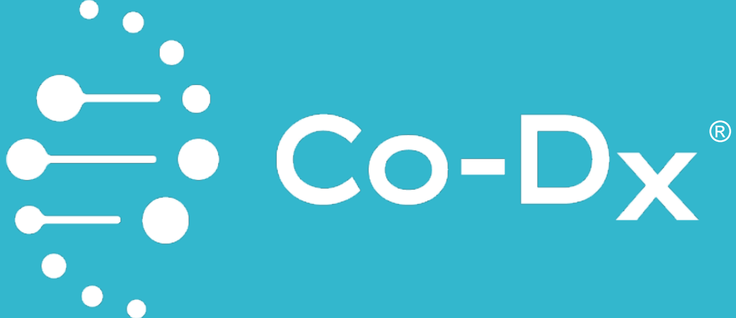

℞ only

Instructions for Use Co-Dx™ Logix Smart® Coronavirus Disease 2019 (COVID-19) Kit

For use under the Emergency use Authorization (EUA) only
For *in vitro* diagnostic use

COVID-K-001-0100
COVID-K-001-0250
COVID-K-001-5000

Co-Dx™ Logix Smart®
Coronavirus Disease 2019 (COVID-19) Kit
CO-DIAGNOSTICS, INC.

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® Coronavirus Disease 2019 (COVID-19)** kit is a real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test intended for the in vitro qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in lower respiratory tract fluids (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), and upper respiratory tract fluids (e.g., nasopharyngeal, anterior nasal, and oropharyngeal swabs) from individuals suspected of COVID-19. A healthcare provider must administer the test. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in lower respiratory tract fluids (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), and upper respiratory tract fluids (e.g., nasopharyngeal, and oropharyngeal swabs) 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 the patient's infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all 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 COVID-19** kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time polymerase chain reaction (qPCR) and in vitro diagnostic procedures. The **Co-Dx Logix Smart COVID-19** kit is only for use under the Food and Drug Administration (FDA) Emergency Use Authorization (EUA).

2 PRODUCT DESCRIPTION AND TEST PRINCIPLE

The **Co-Dx Logix Smart COVID-19** kit is an rRT-PCR test utilizing the Company's patented Co-Primer® technology (Satterfield, 2014) (Poritz & Ririe, 2014). The SARS-CoV-2 Co-Primer sets are designed to detect RNA from SARS-CoV-2 in lower respiratory tract fluids (e.g., bronchoalveolar lavage, sputum, tracheal aspirate) and upper respiratory tract fluids (e.g., nasopharyngeal, and oropharyngeal swabs) from patients who are suspected of COVID-19 by a healthcare provider.

Each **Co-Dx Logix Smart COVID-19** kit consists of the following components:

- Ready-to-use Master Mix (MM), with RNaseP internal positive control (IPC) to verify sample quality
- 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 test begins with the selection of the sample type, followed by a collection of the sample using appropriate procedures and conditions. 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 **Co-Dx Logix Smart® COVID-19** kit assay is a multiplexed, 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 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, anterior nasal, and oropharyngeal swabs).

The purified nucleic acid is then plated with the **Co-Dx Logix Smart COVID-19** MM, 5 µL of each. The MM is pre-mixed and contains the necessary components to perform both the reverse transcription and PCR. This eliminates the need for MM to be prepared ahead of time by the user.

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

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

The 15-minute step at 45°C is the reverse transcription step, where the complementary deoxyribonucleic acid (cDNA) is created from the ribonucleic acid (RNA) template.

The 2-minute step at 95°C is to inactivate the reverse transcriptase and acts as the initial denaturation step for PCR, which is then followed by thermocycling for the PCR.

During the PCR, the fluorescein amidites (FAM) labeled forward Co-Primer acts as both the forward primer and the probe. During the annealing/extension phase of the PCR, the 5’ nuclease activity of Taq polymerase degrades the Co-Primer’s portion that has annealed to the targeted sequence, causing spatial separation between the fluorophore and the quencher, 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.

Components included in the kit are displayed in Table 1.

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Table 1

Kit Components

Cap Color	Component	Symbol	Description	Individual Catalog Number
Brown	**Co-Dx Logix Smart® COVID-19** Master Mix	MM	Proprietary blend of SARS-CoV-2 Co-Primers and PCR reagents	TUBE-CV1-0001 (for 1×500 µL [100 reactions]) TUBE-CV1-0010 (for 1×1,250 µL [250 reactions]) TUBE-CV1-0111 (for 1×25,000 µL [5,000 reactions])
Clear	**Co-Dx Logix Smart COVID-19** Negative Control	NC	Nuclease-free water	TUBE-CV1-0002 (for 1×500 µL [100 reactions]) TUBE-CV1-0020 (for 1×1,250 µL [250 reactions]) TUBE-CV1-0222 (for 1×25,000 µL [5,000 reactions])
Red	**Co-Dx Logix Smart COVID-19** Positive Control	PC	Proprietary blend of SARS-CoV-2 synthetic templates	TUBE-CV1-0003 (for 1×500 µL [100 reactions]) TUBE-CV1-0030 (for 1×1,250 µL [250 reactions]) TUBE-CV1-0333 (for 1×25,000 µL [5,000 reactions])

The product codes are COVID-K-001-0100 for the 100 reaction size, COVID-K-001-0250 for the 250 reaction size, and COVID-K-001-5000 for the 5,000 reaction size. 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

The following list includes information for reagent storage and handling:

- If one or more of the components are not frozen upon receipt or are compromised during shipment, contact your distributor for assistance. The **Co-Dx Logix Smart COVID-19** kit is shipped on dry ice. The components of the kit should arrive frozen.
- Store all components immediately at a temperature between -40 °C and -16°C to prevent degradation of reagents.
- Always work with each **Co-Dx Logix Smart COVID-19** component on ice. Make aliquots, if necessary, to avoid multiple freeze/thaw cycles.

- If you work in an area prone to power outages, keep a back-up generator for your freezer as well as a temperature data log to ensure that the **Co-Dx Logix Smart COVID-19** kit remains frozen at a temperature from between -40°C and -16°C.
- Use the most up-to-date version of this Instructions for Use document found at Product Information - Co-Dx (codiagnostics.com) Stability data for the product is currently being collected and results will be published and available.

4 MATERIAL REQUIRED BUT NOT INCLUDED WITH THE TEST

The extraction reagents, automation systems, and thermal cyclers required but not included with the test are displayed in Table 2, Table 3, and Table 4.

Table 2

Extraction Reagents Validated but Not Included with the Test

Extraction Reagent Options	Catalog Number	Manufacturer
QIAamp Viral RNA Mini Kit	52904 (50 preps), 52906 (250 preps)	Qiagen
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A42352 (200 preps), A48310 (2,000 preps), A48383 (2,000 preps)	Thermo Fisher Scientific
QuickGene Tissue II RNA Kit	RT-S2 (96 preps)	Kurabo

Table 3

Automation Systems Validated but Not Included with the Test

Automation System Options	Catalog Number	Manufacturer
QIAcube	9002864	Qiagen
KingFisher Flex	5400610	Thermo Fisher Scientific
Myra	MYRA	BioMolecular Systems

Table 4

Thermocyclers Validated but Not Included with the Test

Thermocycler	Manufacturer
Co-Dx Box MIC	BMS
7500 Fast Dx, QuantStudio 5 384	ABI

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4.1 Consumables Required but Not Provided

The following consumables are required but not provided:

- Disposable powder-free gloves and lab coats
- Disposable pipette tips with filters
- A 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 equipment is required but is not provided with the kit:

- Several micropipettes capable of pipetting volumes from 5 µL to 1,000 µL
- A cold block or ice
- A vortex and centrifuge
- A PCR workstation, for MM plating and setup

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 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 specific to molecular diagnostics.

Due to the high sensitivity of the **Co-Dx Logix Smart COVID-19** kit and the qPCR technology, handle samples and materials with care while performing the assay to keep reagents and amplification mixtures free of contamination. Users should pay attention to the following:

- Use sterile pipette tips with filters.
- Use standard precautions when handling any patient samples, as they may contain infectious agents.
- Store and extract positive materials (specimens, PCs, and amplicons) separately from other reagents.
- Always use the NC provided with this kit.

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- Consult appropriate Safety Data Sheets (SDS) for instructions on safe use. The SDS for the **Co-Dx Logix Smart COVID-19** kit is provided with the shipment. If not provided with the shipment, the SDS can be retrieved from Co-Diagnostics website at http://co-dx.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 Product Information - Co-Dx (codiagnostics.com)/.

6 SAMPLE COLLECTION, HANDLING, AND STORAGE, SHIPPING

Sample selection, collection, storage, and handling play an essential part in the performance of nucleic acid assays. Valuable information is presented here to help laboratories develop better procedures for the analysis of results and troubleshooting other problems.

For more information, visit the Centers for Disease Control and Prevention (CDC) and the 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

6.1.1 Lower Respiratory Tract Fluids

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

6.1.1.2 **Sputum:** Have the patient rinse their 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 the specimen at a temperature between 2°C and 8°C and ship overnight to the testing laboratory on an ice pack.

6.1.2 Upper Respiratory Tract Fluids

6.1.2.1 **Nasopharyngeal (NP) swab and oropharyngeal (OP) swab:** Use only synthetic fiber swabs with plastic shafts.

Do not use calcium alginate swabs 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 transport media. NP and OP specimens should be kept in separate vials. Collect, store, and ship the sample to the testing laboratory according to appropriate conditions.

> **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.

6.1.2.2 **Oropharyngeal swab (OP) (e.g., throat swab):** Swab the patient's posterior pharynx, avoiding the tongue.

6.1.2.3 **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 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 Handling

Laboratory workers should wear appropriate personal protective equipment (PPE), including 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 that have not been inactivated should be handled within 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 handling 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).

6.3 Sample Storage

Specimen types can be kept at -30°C to -10°C for up to 7 days. For storage longer than 7 days, freeze specimens below -70°C. Avoid repeated freezing and thawing of a specimen. If a specimen is kept for retesting, aliquot the specimen in different tubes to avoid freezing and thawing cycles. Monitor the temperature in the storage areas and record the temperature regularly to identify potential fluctuations.

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

6.4 Sample Shipping

Specimens known to be, or suspected of, containing SARS-CoV-2 that require shipment by air should be shipped as 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). After it is collected and transferred to the clinical lab, the sample will receive an entry into the laboratory system.

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, the following information could 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)

7.1 Sample Preparation

For description, product code, and manufacturer information of the validated reagent kits and automation systems, see Table 1.

7.1.1 Extraction of RNA with QIAamp® Viral RNA Mini Kit, manually or with the QIAcube automation 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 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 in sputum samples.

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.

7.1.2 Extraction of RNA with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit with the KingFisher Flex

7.1.2.1 Prepare the KingFisher Flex Wash plates with a multichannel pipette and covered with MicroAmp clear adhesive film.

7.1.2.1.1 Label a KF 96 DW plate for Proteinase K and pipette 5 µL of Proteinase K solution in each well.

7.1.2.1.2 Label a KF 96 DW plate for Wash 1 and pipette 500 µL of wash solution in each well.

7.1.2.1.3 Label a KF 96 DW plate for Wash 2 and pipette 1000 µL of 80% ethanol in each well.

7.1.2.1.4 Label a KF 96 DW plate for elution buffer and pipette 50 µL of elution buffer in each Elu well.

7.1.2.1.5 Prepare the bulk binding bead solution and ensure that the binding bead solution is homogeneous prior to addition to sample (Pro K) plate.

7.1.2.1.6 Pipette 275 µL of binding bead solution (composed of 265 µL of binding buffer and 10 µL of nucleic acid magnetic beads) in each well being used and pipette to mix.

> **Important:** Do not vortex binding buffer or bead mix solution.

7.1.2.2 Place 200 µL of samples or controls into all appropriate wells of the Proteinase K plate.

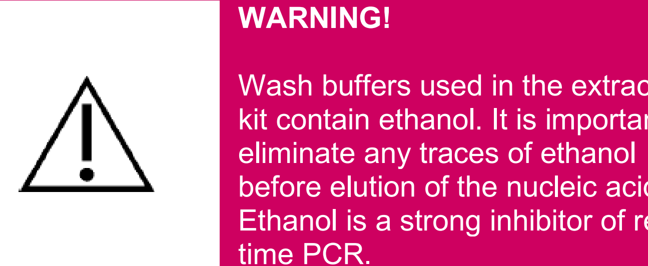
7.1.2.3 Invert the bulk Binding Bead Solution tube about 5 times to ensure the solution is homogeneous prior to dispensing into reagent reservoir. DO NOT VOTEX. Dispense 275 µL of Binding Bead Solution into each of the assigned extraction plate wells, using a fresh tip for each well.

7.1.2.4 Transfer the extraction plate to the KingFisher Flex Instrument for RNA extraction.

7.1.2.5 On the KingFisher Flex, select the MVP_2Wash_200-Flex run protocol from the KingFisher Flex Magnetic Particle Processor menu.

7.1.2.6 Load the plates as prompted by the KingFisher Flex instrument and start the run. When loading each plate, ensure that the plate is oriented so that Well A1 is in the top right corner of the plate holder.

7.1.2.7 After the run is complete (about 22 minutes), remove the Extraction Plate from the instrument. If the plate will not be processed immediately for PCR amplification, seal the plate with a temporary cover and store.



7.1.3 Extraction of RNA with the QuickGene Tissue II RNA Kit with the QuickGene-Mini480.

7.1.3.1 Either follow the manufacturer's instructions [freshly add 10 µL of 2-ME (Sigma-Aldrich) per mL of LRT (Kurabo). Combine 200 µL of LRT + 2-ME, 10 µL of 10 mg/mL Carrier RNA (Sigma-Aldrich), and 150 µL of sample], or use the following optional modification* to buffer LRT:

- Add Carrier RNA to LRT to a final concentration of 10 ng/mL, then immediately prior to use add DTT to a final concentration of 10 mM (Sigma-Aldrich)(LRT*). Combine 200 µL of LRT* with 150 µL of sample.
- Mix sample thoroughly, incubate at room temperature for at least 15 minutes.
- Add 185 µL of solubilization buffer (SRT, Kurabo) and mix thoroughly.

- Add 185 µL of molecular grade ethanol and mix thoroughly.
- Transfer the entire lysate to an extraction column and insert into the QuickGene-Mini480.
- Pressurize the column for at least 15 seconds.
- Add 600 µL of wash buffer (WRT, Kurabo) to each column and pressurize for at least 15 seconds.
- Add 600 µL of wash buffer (WRT, Kurabo) again to each column and pressurize for at least 30 seconds.
- Add 100 µL of elution buffer (Kurabo) to each column and incubate at room temperature for at least 3 minutes.
- Pressurize the column for at least 30 seconds to elute nucleic acids.

7.1.4 Set Up the **Co-Dx Logix Smart COVID-19** Reagent

Follow these steps to set up the reagent:

- 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 **Co-Dx Logix Smart COVID-19** MM, PC, NC, and all sample tubes for about 3 seconds.
- 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.
- Thaw all reagents and samples on ice, or a cold block, before starting the setup.

7.1.5 Set Up the Reaction

Perform the steps below to set up the reaction.

7.1.5.1 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

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

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

7.1.5.2 Pipette 5 µL of MM into each well collected.

7.1.5.3 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.

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

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

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

7.2 PCR Instrument Setup for the Co-Dx Box or MIC

Contact the Laboratory at 801-438-1036 ext. 3 or at www.co-dx.com/contact/ for the template file for download. The template file comes pre-programmed with the PCR instrument setup described in this section. When not using a template, use the settings outlined below to program the Co-Dx Box PCR instrument.

7.2.1 Define the settings displayed in Table 5.

Table 5

Co-Dx Box or MIC PCR Instrument Settings

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

7.2.2 Program PCR instrument with the cycling conditions displayed in Table 6.

Table 6

Co-Dx Box or MIC Cycling Conditions

Stage	Cycles	Temperature	Time
Reverse Transcription	1	45˚C	15 minutes
Initial Denature	1	95˚C	2 minutes
Amplification	50	95˚C	3 seconds
		55˚C	32 seconds

7.2.3 Define the fluorescence detectors (dyes) displayed in Table 7.

Table 7

Co-Dx Box or MIC Fluorescence Detectors (Dyes)

Target	Detector Name	Reporter	Quencher
COVID-19 Specific RNA	COVID-19	FAM™	BHQ® - 1
RNaseP specific DNA (IPC)	RNaseP	CAL Fluor® Red 610	BHQ® - 2

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

7.3 PCR Instrument Setup for the ABI 7500 Fast Dx

7.3.1 Define the settings displayed in Table 8.

Table 8

ABI 7500 Fast System SDS Software v1.4.1 Instrument Settings

Item	Setting
Assay	Standard Curve (Absolute Quantitation)
Container	96-well clear
Template	Blank Document
Sample Volume	10 µL
Run Mode	Fast 7500

7.3.2 Program the PCR instrument with the cycling conditions displayed in Table 9.

Table 9

ABI 7500 Fast System Cycling Conditions

Stage	Cycles	Temperature	Time
Reverse Transcription	1	45˚C	15 minutes
Initial Denature	1	95˚C	2 minutes
Amplification	50	95˚C	3 seconds
		55˚C	32 seconds

7.3.3 Define the fluorescence detectors (dyes) displayed in Table 10.

Table 10

ABI 7500 Fast System Fluorescence Detectors (Dyes)

Target	Detector Name	Reporter	Quencher
COVID-19 Specific RNA	COVID-19	FAM	None
RNaseP specific DNA (IPC)	RNaseP	ROX (or Texas Red)	None
Passive Reference		None	

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

7.4 PCR Instrument Setup for the ABI QuantStudio 5 384

7.4.1 Define the settings displayed in Table 11.

Table 11

ABI QuantStudio 5 384 D&A Software v1.5.1 Instrument Settings

Item	Setting
Instrument Type	QuantStudio 5 System
Block type	384-well block
Experiment Type	Standard Curve
Chemistry	Other
Run Mode	Standard

7.4.2 Program the PCR instrument with the cycling conditions displayed in Table 12.

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Table 12

ABI QuantStudio 5 384 Cycling Conditions

Stage	Cycles	Temperature	Time
Reverse Transcription	1	45˚C	15 minutes
Initial Denature	1	95˚C	2 minutes
Amplification	50	95˚C	3 seconds
		55˚C	32 seconds (Data Collection On)
Sample Volume		10 µL	
Cover		105˚C	

7.4.3 Define the fluorescence detectors (dyes) displayed in Table 13.

Table 13

ABI QuantStudio 5 384 Fluorescence Detectors (Dyes)

Target	Detector Name	Reporter	Quencher
COVID-19 Specific RNA	COVID-19	FAM	None
RNaseP specific DNA (IPC)	RNaseP	ROX	None
Passive Reference		None	

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

8 DATA ANALYSIS

Verification and validation studies performed for **Co-Dx Logix Smart COVID-19** kit were conducted following Good Laboratory Practices for Molecular Biology assays (Viana & Wallis, 2011). If these conditions are not met, the performance will display higher variability, due to user errors while experimenting.

8.1 Co-Dx Box or MIC Analysis Settings

The analysis parameters on the Co-Dx Box or MIC should be set to the following, but after every run, the settings for both the green channel (monitoring for COVID-19), and the orange channel (monitoring for RNaseP [the IPC]), should be verified. To do this, perform the following steps:

- Check the Auto Set Threshold checkbox.
- Set Method to Dynamic.
- Set Threshold Level to 0.100.
- Set Threshold Start to 1.00.
- Set Ignore Cycles Before to 5.
- Set Exclusion to Extensive.

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- Set Fluorescence Cutoff Level to 5.0%.
- Set Initial Y-Axis Scale to Linear.
- Check the box to Auto Generate Analysis.

8.2 ABI 7500 Fast and ABI QuantStudio 5 384 System Analysis Settings

The analysis parameters on the ABI 7500 Fast or ABI QuantStudio 5 384 System should be set to the following and confirmed after every run.

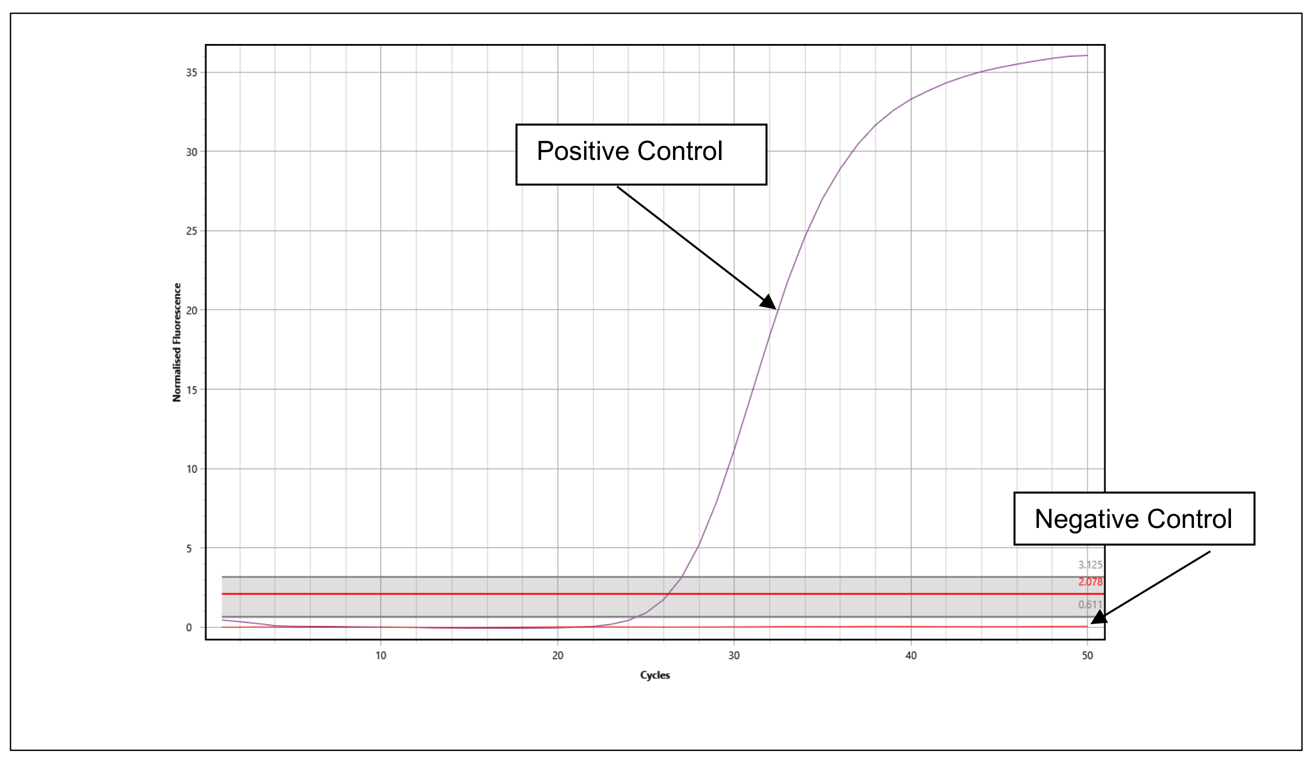
- Recommended **Threshold** 100,000 for both COVID-19 and RnaseP.
- If necessary, the **Threshold** may be manually adjusted so that it is above the background noise and below the plateau.
- **Manual Baseline** Start Cycle 3, End Cycle 15

8.3 Positive Controls (PCs)

Highlight the PC reaction well(s). Each PC should display an amplification curve for the COVID-19 marker in the FAM channel and amplification of the IPC 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 45 cycles.

Figure 1

Positive Control (PC) and Negative Control (NC) Amplification for ***Co-Dx Logix Smart COVID-19***



8.4 Negative Control (NC)

Next, highlight the NC. The results of the NC should show no amplification, specifically with a Cq value less than 45. An example of no amplification can be seen in Figure 1, as the red line, which is below the threshold area (the grey band with the red line).

8.5 The Validity of the Diagnostic Test Runs

Check to see that both the positive and NC have passed.

8.5.1 Ensure the control conditions outlined in Table 14 are met.

Table 14

Control Conditions

Control Type	Control Name	Purpose of Control	COVID-19 FAM Channel	Internal Control (RNaseP) CF610 Channel
COVID-19 Positive Control (PC)	COVID-19 (FAM™)	Verifies the performance of the master mix (MM)	+	+
	RNaseP (CF®610)			
Negative Control (NC)	Nuclease-free water	Verifies the reagents are free of contamination	-	-

8.5.2 If controls pass, interpret the sample results.

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

8.6 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 display an amplification curve or cycle threshold value for COVID-19 at or below 45 cycles. Amplification curves greater than 45 cycles for COVID-19 are in the uncertainty zone. The presence of a curve, with a Cq at or below 45 cycles, for a sample in the COVID-19, indicates a positive result. The amplification of the RNaseP (the IPC) displays that the extraction was successful.

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A negative result will display no amplification for COVID-19 coronavirus; however, occasionally amplification greater than 45 cycles may occur in COVID-19 or RNaseP channels. Any amplification curves greater than 45 cycles for COVID-19 are in the uncertainty zone and could be below the limit of detection. Consider performing a new run of the same sample or another sample from the patient during the following days. The absence of a curve for COVID-19 indicates a negative result ONLY when the RNaseP (the IPC) marker is positive.

An invalid result refers to situations when any of the controls fail. See the Troubleshooting section for details.

Translate the interpretation of results with Ct values according to Table 15.

Table 15

*Interpretation of Results for COVID-19 by Detection of SARS-CoV-2 RdRp Gene with **Co-Dx Logix Smart COVID-19***

	Sample Result		Co-Dx Logix Smart COVID-19 Positive Control (PC)	Co-Dx Logix Smart COVID-19 Negative Control (NC)	Interpretation of Results
	COVID-19 (SARS-CoV-2)	Internal Positive Control (RNaseP) CF610 channel			
Instrument Reading	+	+	+	-	SARS-CoV-2 RNA +
	-	+	+	-	SARS-CoV-2 RNA -
	Any Result (+/-)	-	+	-	INVALID: See Troubleshooting
		+	-	-	
		+	+	+	

An analyte result is positive (+) if the Cq value is <45 or negative (-) if the Cq value is ≥45.

When possible, check that the medical history and/or symptoms match the result before treatment.

9 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Co-Dx Logix Smart COVID-19** kit, even if the recommended steps for troubleshooting solves the issue. To give feedback, fill out the Customer Feedback Form by visiting Customer Feedback - Co-Dx.

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9.1 Stability

Real-time, accelerated shelf-life, and in-use stability studies are currently under testing. With proper storage and handling, the **Co-Dx Logix Smart COVID-19** kit Reagents are stable for up to 12 months from the date of manufacturing.

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

9.2 User Errors

PCR assay is a technique that uses temperature cycling, and a DNA polymerase to amplify a single copy or a few copies of a segment of DNA or RNA. Always use Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) when using this product. This product is not intended to be used by untrained personnel.

The user must have molecular biology experience and must 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 (Centers for Disease Control and Prevention, 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

Invalid results include one or more of the following:

9.3.1 **Co-Dx Logix Smart COVID-19** PC not amplifying

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
- **Co-Dx Logix Smart COVID-19** MM or PC degradation (a result of reagents being at temperatures above -20°C for an extended period)

- The use of an expired reagent
- The use of 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 the PC fails a third time after re-extraction and re-amplification, open a new **Co-Dx Logix Smart COVID-19** PC or MM, and retest. If the PC fails a fourth time, contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 2 or contact us at www.co-dx.com/contact/.

9.3.2 RNaseP (the IPC) Not Amplifying in Patient Samples

A no-amplification result from the RNaseP channel could be the result of one or multiple factors, such as the following:

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

Positive amplification in the COVID-19 channel indicates a positive reading 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.

The results should be interpreted as invalid, and re-testing by re-amplification should be performed. If the IPC fails again, then samples should be re-extracted and re-amplified. If it fails a third time an investigation should be conducted to identify possible causes for the error. If the cause for the error is clear, the test can either be singled out as invalid**,** due to either PCR inhibitors being present, or not enough nuclear material being present. If the cause for an error is unclear, contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 2 or contact us at www.co-dx.com/contact/.

9.3.3 Negative Control (NC) Shows Amplification

Amplification of COVID-19 in the NC 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 NC 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 NC, after re-extraction and re-amplification, happens a third time, open a new NC and retest. If it still fails, the run should be interpreted as invalid. Contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 2 or at: www.co-dx.com/contact/.

10 LIMITATIONS

Limitations include the following:

- 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 www.co-dx.com/resources/instructions-for-use/.
- 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, 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 **Co-Dx Logix Smart COVID-19** 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 kit may fail to detect the presence of the pathogens.
- As with any diagnostic test, results of the **Co-Dx Logix Smart COVID-19** kit are to be interpreted with consideration of all clinical and laboratory findings.
- 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 changes over time.

11 CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The **Co-Dx Logix Smart COVID-19** Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas However, to assist clinical laboratories using **Co-Dx Logix Smart COVID-19** ("your product" in the conditions below), see the following relevant Conditions of Authorization:

- Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets.
- Authorized laboratories using your product will use your product as outlined in the latest version of the Instructions for Use found on the website. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Co-Diagnostics Inc. (phone: +1-801-438-1036 / info@co-dx.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using your product must be appropriately trained in rRT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and must use your product in accordance with the authorized labeling.
- Co-Diagnostics Inc., authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

12 NON-CLINICAL PERFORMANCE EVALUATION

12.1 Limit of Detection (LoD) – Analytical Sensitivity

The Limit of Detection (LoD) refers to the lowest concentration of analyte that is detected at a rate of no less than 95%.

12.1.1 Limit of Detection (LoD) in Sputum using QIAamp Extraction and Co-Dx Box Thermal Cycler

The analytical evaluation of performance was performed with contrived samples produced by spiking in a Genomic RNA of SARS-CoV-2, isolate USA-WA1/2020 (BEI Resources, catalog number NR-52285) in a negative clinical matrix of mainly sputum, bronchoalveolar lavage (BAL), nasopharyngeal fluid, and nasal swab samples acquired from Discovery Life Sciences or donations.

The experiment was performed using genomic RNA of SARS-CoV-2, isolate USA-WA1/2020 (BEI Resources, catalog number NR-52285), which was spiked into sputum samples after the lysis step of the QIAamp Viral RNA Mini Kit (Cat# 52904), to prevent degradation of the RNA before the lysis. The extractions were performed using the QIAcube instrument, with 140 µL of the contrived sample and a 100 µL elution. The extractions were spiked with 10,000; 5,000; 1,000; 600; and 50 total copies after the lysis step. After the extraction process, the extracts were then tested using the **Co-Dx Logix Smart COVID-19** kit protocol. The LoD was confirmed by running at least 20 replicates, at the LoD concentration, which was determined to be 600 total copies. See Table 16 for detailed information.

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Table 16

Genomic RNA Strain SARS-CoV-2 (Isolate USA-WA1/2020) Detection Rate in Sputum

Total Concentration/ Sample	Number of Samples	Number of Detected	Detection Rate (%)	Average Cq	SD (Standard Deviation)	CV% (Coefficient of Variance)
10,000 copies	16	16	100.00	31.55	0.31	0.98
5,000 copies	16	16	100.00	32.22	0.26	0.82
1,000 copies	16	16	100.00	35.06	0.83	2.36
600 copies	16	16	100.00	35.30	0.73	2.08
50 copies	16	1	6.25	38.22	-	-
Positive Control (PC)	2	2	100.00	26.80	0.10	0.38
Negative Control (NC)	6	0	0	0	0	0
Negative extraction (Blank)	8	0	0	0	0	0

After the runs were completed, 600 total copies (4.29 copies/µL in the patient sample) were the lowest concentration with at least a 95% detection rate. Twenty-one replicates, spiked at 600 total copies, were run. The results of that run are displayed in Table 17.

Table 17

Confirmation of the LoD in Sputum

Total Copies/Sample	Number of Samples	Number of Detected	Detection Rate (%)	Average Cq	SD (Standard Deviation)	CV% (Coefficient of Variance)
600 copies	21	21	100.00	35.75	0.66	1.85

The Limit of Detection (LoD) was confirmed to be 600 total copies in 140 µL of sputum, which is a concentration of 4.29 copies/µL or 4,290 copies/mL in the starting contrived patient sample.

12.1.2 Limit of Detection using MagMax Viral/Pathogen Nucleic Acid Isolation Kit on the KingFisher Flex and ABI 7500 Fast Dx thermal cycler

To establish the limit of detection, a serial dilution of a contrived sample was prepared by spiking Accuplex SARS-CoV-2 Verification Panel (SeraCare, 0505-0168) at concentrations of 10,000, 3000, 1000, 500, 100, and 0 genomic copies per mL on clinical matrix of nasal swab in transport media collected from presumed negative subjects.

Samples were extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit extraction using the KingFisher Flex and run on the ABI 7500 Fast Dx Cycler.

After the extraction process, the extracts were then tested using the **Co-Dx Logix Smart COVID-19** kit protocol. The LoD was confirmed by running at least 20 replicates at the LoD concentration, which was determined to be 500 copies/mL as shown in Table 18.

Table 18

Limit of Detection Estimation and Confirmation in Contrived Nasal Swab Samples

Sample Concentration (Copies/ mL)	Number of Samples	Number of Detected	Detection Rate (%)	Average Cq	SD (Standard Deviation)	CV% (Coefficient of Variance)
10,000	9	9	100.00	31.6	0.4	1.4
3,000	9	9	100.00	33.3	0.5	1.6
1,000	9	8	88.89	35.6	1.0	2.9
500	9	8	88.89	35.5	0.7	2.1
100	9	4	44.44	36.6	0.5	1.4
0	9	0	0	N/A	N/A	N/A
Positive Control (PC)	1	1	100.00	25.35	N/A	N/A
Negative Control (NC)	1	0	0	N/A	N/A	N/A
Confirmation of LoD						
500	20	19	95.00	35.9	1.1	3.0
250	20	16	80.00	36.4	0.8	2.2
PC	1	1	100.00	25.35	N/A	N/A
NC	1	0	0	N/A	N/A	N/A

12.1.3 Limit of Detection (LoD) using Kurabo QuickGene Tissue II RNA Kit and MIC Thermal cycler

See Table 19 for Probit-based LoD Summary Data for the MIC thermal cycler. The Limit of Detection was determined using a Probit-based method. Control material obtained from extracted and titrated SARS-CoV-2 viral culture fluid was used for sensitivity and lower limit of detection determination. Quantification was performed using droplet digital PCR (ddPCR) following the manufacturer's instructions (Bio-Rad).

A ten-fold dilution series was first used to determine the detection range. Additionally, linear regression between extrapolated copy number and observed Ct values was performed, as shown in Figure 2, and used to determine an optimal range for a high-resolution titration series crossing the expected LoD. The summary data used to calculate the probit-based limit of detection of 5 copies /reaction (1 copy/µL) are shown in Figure 2.

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Figure 2

95% Lower Limit of Detection and Linear Regression of Cq Versus Copies Using RNA Extracts

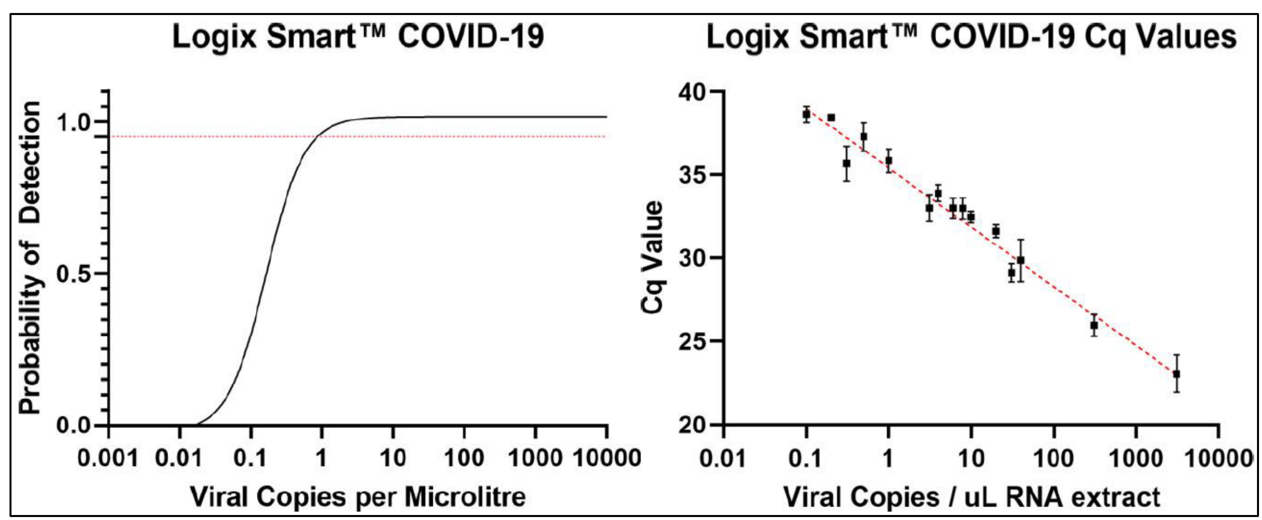

Table 19

Probit-Based LoD Summary Data for the MIC Thermal Cycler

Copies/Reaction	Number of Samples	Number of Detected	Detection Rate (%)	Average Cq
15462.5	10	10	100.00	23.08
1546.25	10	10	100.00	25.96
154.63	10	10	100.00	29.13
15.46	10	10	100.00	33.02
1.54	10	9	90.00	35.66
0.15	10	0	0	N/A
Hi-Resolution Titration Series				
200	10	10	100.00	30.34
100	10	10	100.00	31.64
50	10	10	100.00	32.48
40	10	10	100.00	33.01
30	10	10	100.00	33.02
20	10	10	100.00	33.92
5	10	10	100.00	35.82
2.5	10	10	100.00	37.27
1	10	2	20.00	38.31
0.5	10	6	60.00	38.49
0.15	10	0	0	N/A
Lower Limit of Detection (95%)		5 copies/reaction (upper 95% Confidence Interval = 10.2)		

12.2 Inclusivity (Analytical Sensitivity)

12.2.1 *In Silico* Inclusivity

Alignments have been performed with the oligonucleotide Co-Primer sequences of the COVID-19 Co-Primers with publicly available nucleic acid sequences for SARS-CoV-2 in the GenBank, National Center for Biotechnology Information (NCBI), and the Global Initiative on Sharing all Influenza Data (GISAID) databases to demonstrate the predicted inclusivity of the **Co-Dx Logix Smart COVID-19** kit.

Co-Diagnostics has been performing consistent reviews of the sequence alignment to monitor the sequence conservation by analyzing phylogenetic mutation genomic data pulled by NextStrain from the GISAID database. The first alignment was performed on 1-Feb-2020 with the most recent query performed on 06-Dec-2021 (See Table 20). Sequences were analyzed at https://nextstrain.org/ncov/gisaid/global.

Table 20

Most Recent Monthly In Silico Assessment of NextStrain Sequences from the GISAID Database

Date of Analysis for CoDx's RdRp Marker	Strain	SARS-CoV-2 (sequences in analyzed subsample)	Sequences with 100% homology	Sequences with 1 mismatch (98% homology)	Sequences with 2 mismatches (95% homology)	Sequences with 3+ mismatches (<95% homology)
6-Dec-21	Delta	2135	12 (0.56%)	2116 (99.11%)	7 (0.33%)	0 (0%)
	All Others	1251	1229 (98.24%)	22 (1.76%)	0 (0%)	0 (0%)

The alignment data and posterior updated analyses have shown 95% or greater homology on greater than 99% of publicly available SARS-CoV-2 strains throughout the pandemic for both the forward and reverse Co-Primers on the GISAID database. Therefore, there is <1% prediction of false-negative results, based upon the available data.

Additional in silico analyses have been performed on mutation data for each Variant of Concern (VOC) or Variant of Interest (VOI) lineage including all those displayed in Table 21. No VOC/VOI lineages contain characteristic mutations on the binding targets of our RdRp marker with the exception of the Delta Variant which has undergone further Wet-Test Inclusivity analysis (see section 13.2.2).

Table 21

SARS-CoV-2 Genomic Variants Classified as Variants of Concern or Interest (8-Dec-2021)

Variant of Concern / Interest Status by Agency	WHO Nomenclature	Pango Lineage	NextStrain Clade	Designation	First detected, Date
VOC [WHO, MHRA, CDC]	Alpha	B.1.1.7	20I (V1)	WHO: 18-Dec-20 MHRA: VOC-20DEC-01 CDC: Date not available	UK, Sep-2020
VOC [WHO, MHRA, CDC]	Beta	B.1.351	20H (V2)	WHO: 18-Dec-20 MHRA: VOC-20DEC-02 CDC: Date not available	South Africa, May-2020
VOC [WHO, MHRA, CDC]	Gamma	P.1	20J (V3)	WHO: 11-Jan-2021 MHRA: VOC-21JAN-02	Japan/Brazil, Nov-2020
VOC [WHO, MHRA, CDC]	Delta	B.1.617.2	21A	WHO: VOI-4-Apr-21/ VOC-11-May-21 MHRA: VOC-21APR-02 CDC: VOI-4-May-21 VOC-15-Jun-21	India, Oct-2020
VOC [WHO]	Omicron	B.1.1.529	21K	WHO: 26-Nov-2021	Multiple countries, Nov-2021
VOI [WHO, MHRA, CDC]	Eta	B.1.525	21D	WHO: VOI-17-Mar-2021 MHRA: VUI-21FEB-03	United Kingdom and Nigeria, Dec-2020
VUI [MHRA, CDC]	Zeta	P.2	20B	CDC: VBM-21-Sep-2021 MHRA: VUI-01-Jan-2021	Brazil, Apr-2020
VOI [WHO, CDC]	Iota	B.1.526	21F	WHO: VOI-24-Mar-21 CDC: VOI-21-Apr-21	United States (New York), Nov-2020
VOI [WHO, MHRA, CDC]	Kappa	B.1.617.1	21B	WHO: VOI-4-Apr-2021 MHRA: VUI-21APR-01 CDC: VOI-4-May-21	India, Oct-2020
VOI [MHRA, CDC]	None	B.1.617.3	20A	MHRA: VUI-21APR-03 CDC: VOI-4-May-21	India, Oct-2020
VOI [WHO, MHRA]	Lambda	C.37	21G	WHO: VOI-14-Jun-2021 MHRA: VUI-21JUN-01	Peru, Dec-2020
VOI [WHO, MHRA]	Mu	B.1.621	21H	WHO: VOI-30-Aug-2021 MHRA: VUI-21JUL-1	Colombia, Jan-2021

Abbreviations:
VOC – Variant of Concern
VOI – Variant of Interest
VUI – Variant Under Investigation
VBM – Variant Being Monitored
WHO – World Health Organization
MHRA – Medicines and health Products Regulatory Agency (UK)
CDC – Centers for Disease Control and Prevention (US)

12.2.2 Wet-Test Inclusivity

In the randomized contrived sample study run with the Genomic RNA of SARS-CoV-2, isolate USA-WA1/2020 (BEI Resources, catalog number NR-52285) all positive samples were detected showing 100% detection rate for SARS-CoV-2 (See Table 24).

The sensitivity of the **Co-Dx Logix Smart COVID-19** kit toward Delta variant was empirically evaluated using synthetic RNA oligonucleotides containing either a perfect match Wild-Type (WT) sequence or the single nucleotide Mismatch from the Delta Variant. The acceptance criterion for the experiment was for the LoD of the Mismatch RNA Oligo to be ≤3-fold the LoD of the WT RNA oligo. Both oligos were hydrated with 10% TE buffer to a stock concentration of 1E10 copies/µL, then serially diluted to 1,000, 100, 50, 25, 10, or 5 copies/µL for LoD Estimation.

As shown in Table 22, both RNA oligonucleotides were consistently detected down to 10 or 5 copies/µL, respectively. LoD Confirmation for both Oligos was performed at 10, 7.5, or 5 copies/µL The LoDs of the WT and Mismatch Oligo were confirmed at 10 or 7.5 copies/µL, respectively, meeting the Acceptance Criterion to support retained sensitivity of the **Co-Dx Logix Smart COVID-19** kit toward Delta variant and further corroborating the *in silico* analysis.

Based on these analyses, the **Co-Dx Logix Smart COVID-19** kit retains full sensitivity for lineages classified as VOCs and VOIs at this time, including Alpha, Beta, Gamma, Delta, Omicron, Eta, Zeta, Iota, Kappa, B.1.617.3, Lambda, and Mu, as well as other emerging variant sequences present in the monthly NextStrain sampling of the GISAID database.

Table 22

Delta Variant Synthetic Oligonucleotide LoD Estimation and Confirmation Results

		Wild-Type RNA Oligo			Delta Variant RNA Oligo		
	Copies/µL	Average Ct	S.D.	Number Detected	Average Ct	S.D.	Number Detected
LoD Estimation	10,000	26.14	0.08	2/2	25.50	0	2/2
	1,000	29.07	0.14	2/2	29.17	0.06	2/2
	100	33.06	0.16	4/4	32.64	0.33	4/4
	50	34.36	0.33	4/4	33.63	0.46	4/4
	25	35.08	0.88	4/4	35.27	0.40	4/4
	10	36.02	0.38	4/4	36.69	0.89	4/4
	5	36.50	0	1/2	37.61	0.43	2/2
LoD Confirmation	10	34.83	0.66	20/20	34.63	0.95	20/20
	7.5	37.00	1.12	17/20	36.51	0.84	20/20
	5	37.41	0.80	13/20	36.37	0.68	17/20

12.3 Cross-Reactivity (Analytical Specificity) By an In Silico Analysis

In silico Analysis and 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, and TSA patent sequences; 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 database is reviewed consistently to detect potential mutations in the targeted region.
- 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 alignment is 5 and 2, respectively.
- BLASTn was run individually for every organism requested by the FDA EUA pre-submission process (in silico) guidelines.

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Table 23 displays the list of relevant microorganisms analyzed in the cross-reactivity in silico assessment.

No coronaviruses, other than SARS-CoV-2, or human microflora had any hits with <5 mismatches or >80% total homology that would predict potential false positive rRT-PCR results.

Co-Primers have a slightly different cross-reactivity risk profile than traditional primers. Due to the low Tm's 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 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 COVID-19** kit components do 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 COVID-19** kit to produce positive results.

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Table 23

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	

12.4 Microbial Interference

No microorganism in the in silico analysis has revealed ≥ 80% homology between the cross-reactivity microorganisms, including the ones of relevance listed in Table 23, and the Co-Primers.

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13 CLINICAL EVIDENCE

13.1 Clinical Sensitivity Study using QIAamp Extraction and Co-Dx Box Thermal Cycler in Contrived Sputum Samples

The clinical evidence was established by producing 180 randomized contrived samples spiked with the Genomic RNA of SARS-CoV-2, isolate USA-WA1/2020 (BEI Resources, catalog number NR-52285) in dilutions of 600, 1,000, 5,000, and 10,000 genomic copies per sample (sample input 140 µL). The randomized contrived samples were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, catalog number 52904/52906) and tested with **Co-Dx Logix Smart Coronavirus Disease 2019 (COVID-19)**. The detection rate for **Co-Dx Logix Smart COVID-19** is shown in Table 24. Results also showed consistency of PC results.

Table 24

Randomized Contrived Sputum Sample Detection Rate

Sample Concentration	Number of Samples	Number of Detected	% of Concordant Results (Confidence Interval)	Mean Cq	SD (Standard Deviation)	CV% (Coefficient of Variance)
600 (genomic copies/extraction) (≈ 1× LoD)	9	9	100% (95.6 – 100)	33.21	0.57	1.7
1,000 (genomic copies/extraction) (≈ 2× LoD)	51	51		34.11	0.77	2.3
5,000 (genomic copies/extraction) (≈ 9× LoD)	15	15		31.63	0.34	1.1
10,000 (genomic copies/extraction) (≈ 14× LoD)	15	15		30.59	0.38	1.2
Negative Randomized Contrived Sample	90	0	100% (95.6 – 100)	N/A	100	100

13.2 Clinical Accuracy using MagMax Viral/Pathogen Nucleic Acid Isolation Kit on the KingFisher Flex and ABI 7500 Fast Dx Thermal Cycler

Accuracy runs consisted of 30 positive and 30 negative patient samples identified using the Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 assay. As shown in Table 25, the overall Positive Percent Agreement, Negative Percent Agreement, and Overall Agreement was 100%, with Descriptive Statistics of the Cq values of Positive samples included in the study summarized in Table 26.

Table 25

Clinical Accuracy Summary for MagMax/KingFisher/ABI 7500 Fast Dx

Upper Respiratory Tract Specimens		Rutgers TaqPath SARS-CoV-2 Assay		
		Detected	Not Detected	Total
Co-Dx Logix Smart COVID-19 Assay	Detected	30	0	30
	Not Detected	0	30	30
	Total	30	30	60

Table 26

Cq value Descriptive Statistics for MagMax/KingFisher/ABI 7500 Fast Dx Positives

Statistic	Value
Mean	30.29
Standard Deviation	2.82
Range (Min – Max)	26.15 – 37.29
Interquartile Range	28.30 – 32.26

13.3 Clinical Accuracy for Upper Respiratory Swabs using QuickGene Tissue II RNA Kit and MIC Thermal Cycler

Two cohorts of clinical samples were tested using the **Co-Dx Logix Smart COVID-19** kit on the MIC thermal cycler after extraction with the Kurabo QuickGene Tissue II RNA kit. The specimen types and reference method results for Cohort 1 are shown in Table 27. The specimen types and reference method results for Cohort 2 are shown in Table 28. The primary reference method for both cohorts was a laboratory developed test followed by Sanger sequencing to confirm positives.

Table 27

Sample Summary for Cohort 1

Swab Specimen Type	
Nose and Throat	152
Nasopharyngeal	30
Site Unspecified	24
Throat	1
Total	207

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Table 28

Sample Summary for Cohort 2

Swab Specimen Type	Total
Nose and Throat	217
Nasopharyngeal	3
Throat	4
Total	**224**

13.3.1 Cohort 1 Clinical Accuracy Results

As summarized in Table 29, a total of 207 clinical samples were tested in Cohort 1. Of these, 103 positives were detected by both methods and confirmed by sequencing the Reference Method amplicon. The remaining 104 samples were negative by both methods. The diagnostic accuracy for this study has been calculated and was found to have 100% sensitivity with a confidence interval of 96.48% to 100% and 100% specificity with a confidence interval of 96.52% to 100%. The Positive Predictive Value (PPV) and the Negative Predictive Value (NPV) have both been determined to be at 100%. The accuracy was determined to be 100% with a confidence interval of 98.23% to 100%.

See Table 30 for the Cohort 1 summary statistics.

Table 29

Cohort 1 Clinical Accuracy Results Summary

Upper Respiratory Tract Specimens		Reference Method		
		Detected	Not Detected	Total
Co-Dx Logix Smart COVID-19 Assay	Detected	103	0	103
	Not Detected	0	104	104
	Total	**103**	**104**	**207**

Table 30

Cohort 1 Summary Statistics

Statistic	Total	Confidence Interval
Positive Agreement	100%	96.48 – 100
Negative Agreement	100%	96.52 – 100
Overall Accuracy	100%	98.23 – 100

13.3.2 Cohort 2 Clinical Accuracy Results

Cohort 2 was enriched with additional very weakly positive samples obtained from hotel quarantine (including convalescent patients) analyzed by the Xpert Xpress SARS-CoV-2 test (Cepheid).

As summarized in Table 31, a total of 224 specimens were tested with 97 Detected by both methods, 122 Not Detected by both methods, and 5 Discordant results. The Summary Statistics for Cohort 2 are shown in Table 32, with the Comparative Cq values of concordant versus discordant positives are displayed in Table 33.

The subset of Positive Cohort 2 samples using the Xpert Xpress SARS-CoV-2 test (Cepheid) in addition to (45) or in lieu of (19) the primary reference method exhibited Positive Concordance rates of 42/45 and 17/19, respectively.

Table 31

Cohort 2 Clinical Accuracy Results Summary

Upper Respiratory Tract Specimens		Reference Method		
		Detected	Not Detected	Total
Co-Dx Logix Smart COVID-19 Assay	Detected	97	0	97
	Not Detected	5	122	127
	Total	**102**	**122**	**224**

Table 32

Cohort 2 Summary Statistics

Statistic	Total	Confidence Interval
Positive Agreement	95.1%	88.9 – 98.4
Negative Agreement	100%	97.0 – 100
Overall Accuracy	97.8%	94.9 – 99.3

Table 33

Cohort 2 Reference Method Positive Cq Values

Statistic	Concordant Positives (97)	Discordant Positives (5)
Mean	27.8	35.7
Standard Deviation	5.6	2.7
Range (Min – Max)	13.2 – 43.6	31.3 – 40.8
Interquartile Range	22.8 – 30.7	34.3 – 37.0

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14 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

IVD

CE

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

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16 LEGEND OF PACKAGE SYMBOLS

A legend of the package symbols is displayed in Table 34.

Table 34

Legend of Package Symbols

Icon	Description
IVD	*In vitro* diagnostic medical device
REF	Catalog number
LOT	Batch code
	Use-by-date
Σ x	Contains sufficient for x tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use document
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
℞ only	For prescription use only

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