2020

CO-DIAGNOSTICS INC.

℞ only

Logix Smart™ Coronavirus Disease 2019 (COVID-19) Kit

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

REF **COVID-K-001**

LOGIX SMART™ Coronavirus Disease 2019 (COVID-19) Kit
CO-DIAGNOSTICS, INC.

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1 INTENDED USE

The Logix Smart Coronavirus Disease 2019 (COVID-19) test is a real-time RT-PCR test using a proprietary technology called CoPrimers™ (Satterfield, 2014) (Poritz & Ririe, 2014) intended for the qualitative detection of nucleic acid from the 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 individuals with signs and symptoms of infection who are suspected of having COVID-19. 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, or by similarly qualified non-U.S. laboratories.

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 active infection. 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 Logix Smart Coronavirus Disease 2019 (COVID-19) is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Logix Smart Coronavirus Disease 2019 (COVID-19) test is only for use under the Food and Drug Administration's Emergency Use Authorization.

2 PRODUCT DESCRIPTION AND TEST PRINCIPLE

The Logix Smart Coronavirus Disease 2019 (COVID-19) test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test utilizing the Company's patented CoPrimer technology (Satterfield, 2014) (Poritz & Ririe, 2014). The SARS-CoV-2 CoPrimer sets are designed to detect RNA from the SARS-CoV-2 in lower respiratory tract fluids (e.g. bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract fluids (e.g. nasopharyngeal and oropharyngeal swabs) from patients with signs and symptoms of infection who are suspected of COVID-19.

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

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

2.1 Principles of Operation

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

The Logix Smart COVID-19 test kit assay is a multiplexed single-step real-time reverse transcription PCR test that can be broken down into 3 stages: sample preparation, reverse transcription, and the polymerase chain reaction (PCR) with real-time monitoring. The assay also includes an internal positive control (IPC) that acts as an extraction control to confirm the performance of the extraction.

The sample preparation for PCR requires the samples to be processed to break apart cells and viruses to expose the genetic material. For this process, a commercially available extraction system can be used. In this process, the nucleic acids are isolated and purified from the lower respiratory tract fluids (e.g. bronchoalveolar lavage, sputum, tracheal aspirate), or the upper respiratory tract fluids (e.g. nasopharyngeal and oropharyngeal swabs) using the QIAamp Viral RNA Mini Kit (Qiagen) using the protocol outlined in the product's handbook, "Protocol: Purification of Viral RNA (Spin Protocol)" using 140 µl of the lower respiratory tract fluids (e.g. bronchoalveolar lavage, sputum, tracheal aspirate), or the upper respiratory tract fluids (e.g. nasopharyngeal and oropharyngeal swabs. In the case of sputum samples, the sample should be treated before the extraction by a validated protocol or the CDC's guidelines for "Processing of Sputum Specimens for Nucleic Acid Extraction" (CDC, 2020). Additionally, samples may be processed using the sbeadex Blood Kit (Biosearch Technologies), following the protocol outlined in the product's insert, using 100 µL starting volume, and a 100 µL elution volume (Biosearch Technologies, n.d., p. 6).

The purified nucleic acid is then plated with the Logix Smart Coronavirus Disease 2019 (COVID-19) master mix, 5 µl of each. The master mix is pre-mixed and contains the necessary components to perform both the reverse transcription and PCR and does not need to be prepared ahead of time by the user. The plated reactions will then be put in the thermocycler using the following cycling conditions: 15 min at 45°C, 2 min at 95°C, 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 cDNA is created from the RNA template. The 2 min at 95°C is to inactivate the reverse transcriptase and acts as the initial denaturation step for PCR, which is then followed by the thermocycling for the PCR.

During the PCR, the forward CoPrimer is labeled with FAM and acts as both the forward primer and probe. During the annealing/extension phase of the PCR, the 5' nuclease activity of Taq polymerase degrades the CoPrimer's capture, causing spatial separation between the fluorophore and the quencher, generating a fluorescent signal. With each cycle, additional fluorophore dye 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, specifically the CoDx Box.

Table 2.1 Components included in the test kit

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Black	Logix Smart COVID-19 Master Mix	MM	COVID-MM-001	Proprietary blend of CoPrimers™ and PCR reagents	1x500µL (100 reactions) or 1x1250µL (250 reactions) or 1x25000 µL (5,000 reactions)
Red	Logix Smart COVID-19 Positive Control	PC	COVID-PC-001	Proprietary blend of positive primers	1x500µL (100 reactions) or 1x1250µL (250 reactions) or 1x25000 µL (5,000 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1x500µL (100 reactions) or 1x1250µL (250 reactions) or 1x25000 µL (5,000 reactions)

3 STORAGE AND HANDLING

- The **Logix Smart COVID-19** kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more of the components are not frozen upon receipt or are compromised during shipment, contact your distributor for assistance.
- All components should be stored immediately at or below -20°C to prevent degradation of reagents.
- Always work with each **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 it is recommended to have a back-up generator for your freezer as well as a temperature data log to ensure that the **Logix Smart COVID-19** test kit remains frozen at -20°C.
- Stability data for the product is currently being collected and results will be published, and new Instructions for Use updated to reflect the stability conditions.

4 MATERIAL REQUIRED BUT NOT INCLUDED WITH THE TEST

Extraction System required but not included with the test:

Table 4.1 Extraction systems validated with the test

Extraction System Options	Catalog number	Manufacturer
QIAamp Viral RNA Mini Kit	52904 (50 extractions) 52906 (250 extractions)	Qiagen
sbeadex Blood Kit	NAP44401 NAP44410 NAP44100	Biosearch Technologies

Table 4.2 Thermocyclers validated but not included with the test

Thermocycler Machine	Manufacturer
CoDx Box	BMS, Bio Molecular Systems
MIC qPCR Cycler	BMS, Bio Molecular Systems
Eco 48	PCRmax

4.1 Consumables required but not provided:

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

4.2 Equipment required but not provided:

- Several micropipettes capable of pipetting volumes from 5µL to 1000µL
- A cold block or ice
- Vortex and centrifuge
- Class II Biosafety cabinet, ideally in a BSL-2 containment facility, for the extraction
- PCR workstation, for master mix plating and setup
- CoDx Box (Bio Molecular Systems, distributed by Co-Diagnostics, Inc.)
- qPCR thermocycler with channels capable of detecting FAM and CF610 fluorophores.

5 WARNINGS AND PRECAUTIONS

WARNING!

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

As with any diagnostic or laboratory experiment, good laboratory practices for molecular biology is essential to the proper performance of the qPCR or any laboratory experiment. Attention should be taken to the procedures particular to the molecular diagnostics procedures. Due to the high sensitivity of **Logix Smart COVID-19** and the qPCR technology, care should be taken while handling samples and materials while performing the assay to keep reagents and amplification mixtures free of contamination. Users should 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, positive controls, and amplicons) separately from other reagents.
- Always use nuclease-free water, provided with this kit.
- Consult appropriate Safety Data Sheets (SDS) for safety. The SDS for the **Logix Smart COVID-19** test kit is provided with the shipment. If not provided with the shipment, the SDS can be retrieved from Co-Diagnostics website at the link: http://codiagnostics.com/products/diagnostic-solutions/
- To prevent contamination, it is required to use Good Laboratory Practices for Molecular Biology, which requires a unidirectional workflow and the separation of negative and positive materials.
- Please, always use the most recent version of this document as more information is added with future studies. This can be downloaded for free at http://codiagnostics.com/resources/instructions-for-use/

6 SAMPLE COLLECTION, TRANSPORT, AND STORAGE

The sample selection, collection, storage, and handling play an essential part in the performance of nucleic acid assays. Thus, 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 CDC's and WHO's websites in the following addresses:

a) Lower respiratory tract fluids
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 2-8°C and ship overnight to the testing laboratory on an ice pack.

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

b) Upper respiratory tract fluids
Nasopharyngeal swab AND oropharyngeal swab (NP/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 2-3 mL of viral transport media. NP and OP specimens should be kept in separate vials. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack.

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

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

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

6.1 Sample Handling

Laboratory workers should wear appropriate personal protective equipment (PPE), which includes disposable gloves, laboratory coat/gown, and eye protection when handling potentially infectious specimens.
Clinical specimens from patients suspected or confirmed to be infected with COVID-19 should be conducted under a certified class II biosafety cabinet in a BSL-2 containment facility. More details are provided in the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (CDC, 2009) or the *WHO Laboratory Biosafety Manual* (WHO, 2004).

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

6.2 Sample Storage

It is recommended that all specimen types, besides whole blood, be kept at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C. Repeated freezing and thawing of a specimen should be avoided. If a specimen is kept for retesting, it should be aliquoted in different tubes to avoid freezing and thawing cycles. The temperature in the storage areas should be monitored and recorded regularly to identify potential fluctuations. Domestic refrigerators/ freezers with wide temperature fluctuations are not suitable for the storage of frozen specimens (CDC, 2020).

6.3 Sample Shipping

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

7 PROCEDURE

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

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

7.1 Sample Preparation

The quality of the RNA from the extraction of the sample is essential to the performance of **Logix Smart COVID-19**. The extraction protocol should be performed following the manufacturer's instructions or an internally validated protocol. However, 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).

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the other nucleic acid extraction procedure for use with **Logix Smart COVID-19** must be validated by the user.

Extraction of RNA using the QIAamp® Viral RNA Mini Kit must be performed following the manufacturer's instructions using 140 µL of the sample, and a modified elution using 100 µL of buffer AVE. It is highly recommended before the elution of nucleic acids to ensure the removal of all ethanol. For column-based kits that include washing with buffers containing ethanol, an additional centrifugation step (see extraction procedure) using a new collection tube is recommended.

If your sample preparation system uses wash buffers containing ethanol, make sure to eliminate any traces of ethanol before elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

The use of carrier RNA can be crucial for extraction efficiency and stability of the extracted nucleic acid.

7.2 Logix Smart COVID-19 Reagent Setup

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by molecular grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.
- All **Logix Smart™ COVID-19** Master Mix, Positive Control (PC), nuclease-free water (used as a no template control or NTC), and sample tubes should be vortexed for 3 seconds and briefly spun down before using to ensure properly mixed reagents and to remove any condensation or residue from the lids.
- Thaw all reagents and samples on **ice**, or a cold block, before starting setup.

7.3 Reaction Set Up

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

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

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

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

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

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

7.4 PCR Instrument Setup

7.4.1 If using Co-Diagnostics Inc. CoDx Box, contact the Laboratory 801-438-1036 ext. 04 or at www.codiagnostics.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, or when using another device, use the settings outlined below to program the PCR instrument.

7.4.2 To achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined below.

7.4.3 Define the following settings:

Reaction Volume	10 µL
Ramp Rate	Default
Passive Reference	None

7.4.1 Program PCR instrument with the cycling conditions below:

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

7.4.2 Ensure that the PCR instrument being used is compatible with fluorophores below. Some devices may not have options for the quencher. If help is needed or in case of questions, contact Co-Diagnostics Inc. technical support at 801-438-1036 ext. 04 or at: www.codiagnostics.com/contact/.

7.4.3 Define the fluorescence detectors (dyes):

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

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

8 DATA ANALYSIS

For basic information regarding data analysis on a specific real-time PCR instruments, please refer to the user manual of the respective instrument.

Verification and validation studies performed for **Logix Smart™ Coronavirus Disease 2019 (COVID-19) (COVID-K-001)** 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 The Validity of the Diagnostic Test Runs

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

8.1.1 The following control conditions must be met:

Control Type	Control Name	Purpose of Control	COVID-19 FAM channel	Internal Control (RNaseP) CF610 channel
COVID-19 Positive Control	COVID-19 (FAM™)	Verifies the performance of the master mix	+	+
	RNaseP (CF®610)			
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-

- If controls pass, interpret the sample results.

8.1.2 Invalid Diagnostic Test Run

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

8.2 Interpretation of Results

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

- Positive
- Negative
- Inconclusive

A **Positive** result will show 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 (IPC) shows that the extraction was successful.

A **Negative** result will show 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 possibly below the limit of detection. New run of the same sample or run of another sample of the patient in the same of following days should be considered. The absence of a curve for COVID-19 indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

An **Inconclusive** result refers to situations when any of the controls fail. See troubleshooting.

The interpretation of results with Ct values can be translated to the following table:

Table 8.1 Interpretation of Results for COVID-19 by detection of SARS-CoV-2 RdRp gene with Logix Smart COVID-19

	Sample Result		Logix Smart™ COVID-19 Positive Control	No Template Control (NTC) (Master Mix + Water)	Interpretation of Results
	COVID-19 (SARS-CoV-2)	Internal Positive Control (RNaseP) CF610 channel			
Instrument Reading	+	+	+	-	COVID-19 +
	-	+	+	-	COVID-19 -
	Any Result (+/-)	-	+	-	Inconclusive: See Troubleshooting
		+	-	-	
		+	+	+	

Anything before 45 cycles is considered a positive reading (+). Anything after 45 cycles is considered a negative reading (-). When possible, always 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 **Logix Smart™ COVID-19** test kit**,** even if the recommended steps for troubleshooting solves the issue. To give feedback please fill out the Customer Feedback Form by visiting codiagnostics.com/contact/feedback/

9.1 Stability

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

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

9.2 User Errors

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

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

A 90 minutes online training for Good Laboratory Practices for Molecular Genetics Testing (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/ Inconclusive Results

9.3.1 **Logix Smart COVID-19 Positive Control** not amplifying

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

- 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,
- **Logix Smart COVID-19 Master Mix** or **Logix Smart COVID-19 Positive Control** degradation (a result of reagents being at temperatures above -20°C for an extended period),
- Use of expired reagents,
- or the wrong reagents being used.

Without further evidence, it is best to disregard the results from the patient samples and re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the patient samples may need to be re-extracted. If failure of the positive control happens a third time after re-extraction and re-amplification, open a new **Logix Smart COVID-19 Positive Control** or **Master Mix**, and retest. If still failing, please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or contact us at www.codiagnostics.com/contact/.

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:

- Not enough nuclear material in the patient sample,
- PCR inhibitors such as ethanol and heparin,
- the extraction was performed incorrectly,
- or the extraction kit used is not compatible or has a step that eliminates RNaseP DNA.
- Note: Positive amplification in the COVID-19 channel indicates a positive result despite the lack of concurrent amplification in the IPC channel. The IPC amplification is dependent on the presence of human genomic DNA (gDNA) in the extraction sample, the amount of which is governed by the type of the patient sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g. CSF, urine, cell lysates, etc.) may not contain the human RNaseP gene.

Negative patient results cannot be trusted 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 **inconclusive** 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. 04 or contact us at www.codiagnostics.com/contact/.

9.3.3 **No Template Control** showing amplification

- Amplification of COVID-19 in the No Template Control indicates contamination of one or more of the reagents, incorrect placement of plate or tube into the real-time PCR instrument, or pipetting errors.

None of the results can be trusted and re-testing by re-amplification should be performed. If the NTC fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the patient samples may need to be re-extracted. If failure of the NTC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or at: www.codiagnostics.com/contact/.

10 LIMITATIONS

- Strict compliance with this document is required for optimal results. Please, always use the most recent version of this document. This can be downloaded for free at codiagnostics.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 that a test run be performed to check the performance of the reagents before testing on patient samples.
- Appropriate specimen collection, transport, storage, and processing procedures are required for optimal results.
- Do not use the **Logix Smart 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 test kit may fail to detect the presence of the pathogens.
- As with any diagnostic test, results of the **Logix Smart COVID-19** kit are to be interpreted with consideration of all clinical and laboratory findings.

11 NON-CLINICAL PERFORMANCE EVALUATION

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) or synthetic RNA template of SARS-CoV-2 (IDT, a custom template containing targeted region) in a negative clinical matrix of sputum, bronchoalveolar lavage (BAL), nasopharyngeal fluid, and nasal swab samples acquired from Discovery Life Sciences or donations.

11.1 Limit of Detection (LoD) – Analytical Sensitivity

The Limit of Detection (LoD) study has the purpose of identify the lowest detectable concentration of SARS-CoV-2 in a given sample with 95% confidence, which means 95% or greater confidence of finding true positive in all replicates.
The experiment was performed using an in vitro transcribed multi-gene RNA spiked into a clinical matrix (sputum) to create serial dilutions of 10, 8, 5, 3, 0.5 copies per reaction. The samples were extracted using the QIAcube instrument with the QIAamp Viral RNA Mini Kit (Cat# 52904). The extracts were then tested using the Logix Smart COVID-19 test kit protocol described on its Instructions for Use. The detection rate is displayed in Table 11.1.

Table 11.1 SARS-CoV-2 Multi gene transcribe Detection Rate in sputum

Sample Concentration	# of Samples	# of Detected	Detection Rate (%)	Average Cq	SD (Standard Deviation)	CV% (Coefficient of Variance)
10 copies/reactions	20	19	95.00	37.10	0.30	0.8
8 copies/reactions	20	20	100.00	37.10	0.11	0.3

5 copies/reactions	20	14	70.00	37.94	0.27	0.72
3 copies/reactions	20	9	45.00	37.91	0.55	1.45
0.5 copies/reactions	19	4	21.05	37.89	0.50	1.32
Positive Control (PC)	3	3	100.00	27.34	0.14	0.52
Nuclease-Free Water	9	0	0	0	0	0
Negative extraction (Blank)	12	0	0	0	0	0

The Limit of Detection (LoD) was determined by Probit Analysis to 9.35 copies/µL or $9.35x10^3$ copies/mL.

11.2 Inclusivity (analytical sensitivity):

11.2.1 In silico inclusivity

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

Co-Diagnostics has been performing consistent reviews of the sequence alignment to monitor the sequence conservation by analyzing phylogenic mutation genomic data pulled by NextStrain from the GISAID database. Sequences were obtained from https://github.com/nextstrain/ncov/blob/master/data/metadata.tsv

The alignment data and posterior updated analyses have shown a 100% identity for both the forward and reverse CoPrimers on the GISAID database. Therefore, there is no prediction of false-negative results based upon the available data.

11.2.2 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.

11.3 Cross-reactivity (Analytical Specificity) by an *in silico* analysis:

In silico Analysis and BLASTn analysis queries of the SARS-CoV-2 CoPrimers were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database is reviewed consistently to detect potential mutations in the targeted region; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in alignment is 5 and 2 respectively. 7) BLASTn was run individually for every organism requested by the FDA EUA pre-submission process (in silico) guidelines. Table 11.2 shows the list of the relevant microorganisms analyzed in silico.

Table 11.2 Microorganism included in the cross-reactivity in silico assessment

Microorganisms	
Influenza C	*Neisseria meningitides*
Parechovirus	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus epidermidis*
Corynebacterium diphtheriae	*Streptococcus salivarius*
Legionella non-pneumophila	Leptospirosis
Bacillus anthracis (Anthrax)	*Chlamydia psittaci*
Moraxella catarrhalis	*Coxiella burnetii* (Q-Fever)
Neisseria elongata	*Staphylococcus aureus*

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

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

The results suggest that the **Logix Smart COVID-19** does not cross-react to any of the non-target organisms that were tested in the wet test or *in silico* analysis. The negative samples did not show any amplification, therefore, no false positives occurred due to cross-reactivity. Positive samples in the presence of non-target organism genetic material in most cases did not reduce the ability of the **Logix Smart COVID-19** test to produce positive results.

11.4 Cross-reactivity (Analytical Specificity) by a Wet-test:

The Logix Smart COVID-19 test kit was tested against multiple non-target relevant organisms in a wet test. The non-target organisms that were used are listed in Table 11.3. The test was performed by spiking negative matrices (serum, bronchoalveolar lavage (BAL), and nasopharyngeal fluid) with non-target organisms, or the non-target organism's extracted genome.

Table 11.3 Microorganisms reference material used verification and validation testing

Microorganism	Supplier	Catalog Number	Strain
Coronavirus RNA	Vircell	MBC090-R	HCoV-229E (ATCC VR-740)
Coronavirus OC43 RNA	Vircell	MBC135-R	HCoV-OC43
MERS Coronavirus RNA	Vircell	MBC132-R	MERS-CoV (England-1)

Total Respiratory Viral Panel Control (Swab)	Vircell	MBT020	Adenovirus 4, Coronavirus, Influenza A H3N2, Influenza B, Novel influenza A H1N1, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Respiratory syncytial virus (subtype A), and Respiratory syncytial virus (subtype B).
Haemophilus influenzae DNA	Vircell	MBC020-R	Haemophilus influenza B (strain ATCC 33533 BexA), and *Haemophilus ducreyi* (strain CIP542)
Enterovirus 68 RNA	Vircell	MBC056	F02-3607 (Corn strain)
Rhinovirus RNA	Vircell	MBC091-R	1059
Mycobacterium tuberculosis DNA	Vircell	MBC034	H37Rv
Bordetella pertussis DNA	Vircell	MBC008-R	Strain F
Mycoplasma pneumoniae DNA	Vircell	MBC035-R	Strain FH of Eaton Agent
Chlamydophila pneumoniae	ATCC	VR-2282	Twar strain TW-183
Legionella pneumophila subsp. pneumophila	ATCC	33152DQ	ATCC 33512DQ
Middle East respiratory syndrome coronavirus (MERS-CoV) RNA	ATCC	VR-3248SD	ATCC VR-3248SD
Human coronavirus HKU1 RNA	ATCC	VR-3262SD	HKU1 (VR-3262SD)
Human coronavirus (HCoV)	BEI Resources	NR-44105	NL63
Human metapneumovirus (hMPV) Nasal swab culture positive	Discovery Life Sciences	Not available	

The materials that were already extracted were spiked into negative serum extract. The materials that needed to be extracted were spiked in before the extraction. Table 11.2 lists the non-target organisms used in cross-reactivity testing. Non-target organisms were spiked in at a final concentration of 1e4 copies/reaction and run in triplicate.

Additionally, to verify that the presence of non-target genomic DNA doesn't affect the ability of Logix Smart COVID-19 to detect SARS-CoV-2, non-target organisms were spiked in at a final concentration of 1e4 copies/reaction in conjunction of the SARS-CoV-2 template, which was spiked at a concentration that would result in a 99% detection rate, and run in triplicate. The concentration of 1e4 copies/reaction was chosen as a result of the limited amount of materials supplied in the reference material.

Table 11.4 Wet-test for cross-reactivity in negative matrix and negative matrix spiked with SARS-CoV-2 RNA template in the presence of other organisms

Non-target Organism	Final Concentration of the Non-target organism	Non-target Organism in the Negative Matrices Below	Non-target Organism + SARS-CoV-2 Template
Vircell		**Serum, Sputum, Nasopharyngeal Fluid, and Bronchoalveolar Lavage**	**Serum, Sputum, Nasopharyngeal Fluid, and Bronchoalveolar Lavage**
Amplirun Coronavirus RNA	1e4 copies/ reaction	Negative	Positive
Amplirun Coronavirus OC43 RNA	1e4 copies/ reaction	Negative	Positive
Amplirun MERS Coronavirus RNA	1e4 copies/ reaction	Negative	Positive
Amplirun Total Respiratory Viral Panel	1e4 copies/ reaction	Negative	Positive
Amplirun Enterovirus DNA	1e4 copies/ reaction	Negative	Positive
Amplirun Rhinovirus RNA	1e4 copies/ reaction	Negative	Positive
Amplirun *Haemophilus Influenzae* DNA	1e4 copies/ reaction	Negative	Positive
Amplirun *Mycobacterium tuberculosis* DNA	1e4 copies/ reaction	Negative	Positive
Amplirun *Bordetella pertussis* DNA	1e4 copies/ reaction	Negative	Positive
Amplirun *Mycoplasma pneumoniae* DNA	1e4 copies/ reaction	Negative	Positive
ATCC			
Chlamydophila pneumoniae	1e4 copies/ reaction	Negative	Positive
MERS coronavirus	1e4 copies/ reaction	Negative	Positive
Human coronavirus HKU1	1e4 copies/ reaction	Negative	Positive
Legionella pneumophila	1e4 copies/ reaction	Negative	Positive
BEI Resources			
Human coronavirus NL63	1e4 copies/ reaction	Negative	Positive
IDT			
Positive SARS-CoV-2 RNA Sample	1e4 copies/ reaction	Negative	Positive

11.5 Microbial Interference

Wet testing has been performed with relevant microorganisms when the RNA or inactivated microorganism is commercially available. No microorganism in the *in silico* analysis has revealed ≥ 80% homology between the cross-reactivity microorganisms and the CoPrimers.

12 CLINICAL EVIDENCE

The clinical evidence has been performed 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 60, 100, 500, and 1,000 genomic copies per extraction. The randomized contrived samples were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, catalog number 52904) and tested with **Logix Smart Coronavirus Disease 2019 (COVID-19)**. The detection rate for Logix Smart COVID-19 is shown in Table 12.1. Results also showed consistency of positive control results. Sensitivity and specificity are disclosed in Table 12.2.

Table 12.1 Randomized Contrived Sample Detection Rate

Sample Concentration	# of Samples	# of Detected	% of Positive Results (Confidence Interval)	Mean Cq	SD (Standard Deviation)	CV% (Coefficient of Variance)
60 (genomic copies/extraction) (≈ 1x LoD)	9	9	100 (CI 86.7 – 100)	33.21	0.57	1.7
100 (genomic copies/extraction) (≈ 2x LoD)	51	51	100 (CI 86.7 – 100)	34.11	0.77	2.3
500 (genomic copies/extraction) (≈ 9x LoD)	15	15	100 (CI 86.7 – 100)	31.63	0.34	1.1
1,000 (genomic copies/extraction) (≈ 14x LoD)	15	15	100 (CI 86.7 – 100)	30.59	0.38	1.2
Negative Randomized Contrived Sample	90	0	0 (Not Applicable)	Not Applicable	0	0

Table 12.2 Detection Rate for Logix Smart COVID-19

Diagnostic Accuracy	Results for Logix Smart COVID-19
Number of True Negatives (TN)	90
Number of False Positives (FP)	0
Number of True Positives (TP)	90
Number of False Negatives (FN)	0
Sensitivity	100%
Specificity	100%
Positive Predictive Value (PPV)	100
Negative Predictive Value (NPV)	100
Mathews Correlation Coefficient (MCC)	1.00

13 MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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IVD

CE

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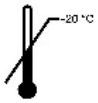
15 LEGEND OF PACKAGE SYMBOLS

IVD	*In vitro* diagnostic medical device		Protect from light
REF	Catalog number		Temperature limit
LOT	Batch Code		Consult Instructions for Use
CAP	Cap color		Non-Sterile product – Do not sterilize
COMP	Component		Manufacturer
CONT	Content/Volume	EC REP	Authorized Representative in the European Community
NUM	Number	CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC
	Use-by-date	℞ only	For prescription use only
 100	Contains sufficient for 100 tests/reactions		