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Logix Smart™ SARS-CoV-2 DS (COVDS-K-003)

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

REF

COVDS-K-003

Logix Smart™ SARS-CoV-2 DS
CO-DIAGNOSTICS, INC.

*Co-Diagnostics has filed for Emergency Use Authorization with the US FDA. This test has been validated by Co-Diagnostics, but the US FDA's independent review of this validation is pending.

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1 INTENDED USE

The Logix Smart SARS-CoV-2 DS is a real-time multiplex RT-PCR test, using a proprietary primer technology called CoPrimers™, intended for the *in vitro* qualitative detection of nucleic acid from SARS-CoV-2, targeting the *RdRp* and *E* genes, in saliva collected without preservatives in a sterile container from individuals suspected of COVID-19 by their healthcare provider. The Logix Smart SARS-CoV-2 DS does not require the conventional extraction process utilizing columns or beads, but instead amplifies the targeted nucleic acid after a brief treatment of the sample with Proteinase K. 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 saliva during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities. Many laboratories are required to report all positive results to the appropriate public health authorities.

The Logix Smart SARS-CoV-2 DS is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Logix Smart SARS-CoV-2 DS test is only for use under the Food and Drug Administration's Emergency Use Authorization.

The Logix Smart SARS-CoV-2 DS has been validated by Co-Diagnostics, but the US FDA's independent review of this validation is pending.

2 PRODUCT DESCRIPTION AND TEST PRINCIPLE

The Logix Smart SARS-CoV-2 DS is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test utilizing the Co-Diagnostics' patented CoPrimer technology (Satterfield B. , 2014) (Poritz & Ririe, 2014). The two sets of CoPrimers are designed to detect RNA from SARS-CoV-2 in saliva collected without preservatives in a sterile container from patients who are suspected of COVID-19. This test uses a sample processing technique that eliminates the use of the nucleic acid extraction/purification process.

Each **Logix Smart SARS-CoV-2 DS** 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

Traditional nucleic acid extraction purifies and isolates nucleic acid from the sample matrix. In contrast, the sample preparation protocol for Logix Smart SARS-CoV-2 DS bypasses traditional extraction and releases nucleic acids in saliva collected without preservatives in a sterile container with a brief treatment with Proteinase K. A 50 µL of saliva is combined with 2.5 µL of Proteinase K (20mg/mL). This mixture is vortexed for 1 minute, followed by heating at 95 °C for 5 minutes to deactivate Proteinase K. 10 µL of the processed sample is then plated with 10 µL of Logix Smart SARS-CoV-2 DS master mix. The master mix is pre-mixed and contains the necessary components to perform both the reverse transcription converting the RNA into cDNA which is then subsequently amplified in the PCR by one of the approved thermocyclers. The master mix does not need to be prepared ahead of time by the user.

The following cycling conditions are used for the reverse transcription and PCR steps: 15 min at 45°C, 2 min at 95°C, 45 cycles x [3s at 95°C, 32s at 55°C]. The 15-minute step at 45°C is the reverse transcription step, where the cDNA is created from the RNA template. The 2 min at 95°C is to inactivate the reverse transcriptase, and acts as the initial denaturation step for PCR, which is then followed by the cycling for the PCR.

In the PCR process, the capture portion of the CoPrimer anneals to a specific target sequence of the virus while the priming sequence of the CoPrimer anneals to another region downstream from the capture sequence (Satterfield B. C., 2014). During the extension phase of the PCR, the 5' nuclease activity of the polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. A complementary strand of DNA is also created. In the Logix Smart SARS-CoV-2 DS test the CoPrimers are labeled with FAM (green) and Cal Flour Red 610 (orange). With each cycle, additional reporter dye molecules are cleaved from their respective capture or priming sections, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the thermocycler.

The Logix Smart SARS-CoV-2 test kit is offered in two presentations displayed in **Table 2.1**

Table 2.1 Logix Smart SARS-CoV-2 Kit Presentations

Component	Catalog Number	Description	Size	Kit Components	Part Codes
Logix Smart SARS-CoV-2 DS	COVDS-K-003	Test kit with 3 tubes	1x1,000 µL (100 reactions) or 1x25,000 µL (2,500 reactions)	Logix Smart SARS-CoV-2 DS Master Mix	COVDS-MM-003
				Logix Smart SARS-CoV-2 DS Master Mix	COVDS-PC-003
				Nuclease Free Water	GEN-NF-001
	COVDS-K-003-01	Test kit with 4 tubes	1x1,000 µL (100 reactions) or 1x25,000 µL (2,500 reactions)	Logix Smart SARS-CoV-2 DS Master Mix	COVDS-MM-003
				Logix Smart SARS-CoV-2 DS Master Mix	COVDS-PC-003
				Nuclease Free Water	GEN-NF-001
				CoDx Processing Solution	SPS-DSR01

Table 2.2 Logix Smart SARS-CoV-2 DS Kit Components

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Brown	Logix Smart SARS-CoV-2 DS Master Mix	MM	COVDS-MM-003	Proprietary blend of SARS-CoV-2 CoPrimers™ and PCR reagents	1x1000 µL (100 reactions) or 1x25000 µL (2,500 reactions)
Red	Logix Smart SARS-CoV-2 DS Positive Control	PC	COVDS-PC-003	Proprietary blend of SARS-CoV-2 synthetic templates	1x1000 µL (100 reactions) or 1x25000 µL (2,500 reactions)

Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1x1000 µL (100 reactions) or 1x25000 µL (2,500 reactions)
	CoDx Processing Solution*	SPS	SPS-DSR01	Proteinase K solution 20 mg/mL	1x2,500 µL (100 reactions) or 1x6,250 µL (2,500 reactions)

*CoDx Processing Solution is only offered in the kit with ordering code COVDS-K-003-01 and is not available in the ordering code COVDS-K-003.

3 REAGENT STORAGE AND HANDLING

- The **Logix Smart SARS-CoV-2 DS** test 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 SARS-CoV-2 DS** 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 SARS-CoV-2 DS** 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 KIT

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

4.2 Sample Processing Reagent

The Logix Smart SARS-CoV-2 DS is offered in two presentations to provide customers better flexibility on the use of supplies. Laboratories opting to use the Logix Smart SARS-CoV-2 DS, product code COVDS-K-003, will not receive a Proteinase K solution with the kit and may instead use the alternative Proteinase K validated with the kit and displayed in **Table 4.1** below.

Table 4.1 Alternative Proteinase K Solution Validated but Not Included with the Test

Sample Treatment Reagent	Manufacturer	Catalog #	Volume Added to Sample
MagMAX™ Viral/Pathogen Proteinase K	Thermo Fisher Scientific	A42363	2.5 µL

4.3 Equipment required but not provided:

- Several micropipettes capable of pipetting volumes from 5 µL to 1000 µL
- A cold block or ice
- Vortex and centrifuge
- Class II Biosafety cabinet, ideally in a BSL-2 containment facility, for sample processing
- Heat block capable of reaching 95°C
- PCR workstation, for master mix plating and setup
- Thermocycler (**Table 4.2**)

Table 4.2 Thermocyclers Validated but Not Included with the Test

Thermocycler Machine	Catalog Number	Manufacturer	Number of wells
CoDx Box Cycler	CDX-MC4	Co-Diagnostics, Inc.	48
Mic qPCR Cycler	MIC-4	BMS, Bio Molecular Systems	48
QuantStudio™ 5 Real-Time PCR System	A34322	Applied Biosystems (Thermo Fisher Scientific)	96
CFX 96 Touch Real-Time PCR Detection System	1855195	Bio-Rad	96
Applied Biosystems 7500 Fast Dx Real-Time Instrument	4406984	Thermo Fisher Scientific (Applied Biosystems)	96

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.

"Logix Smart SARS-CoV-2 DS Test has not been cleared or approved; the test is pending authorization by US FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvements Act (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests"

As with any diagnostic or laboratory experiment, good laboratory practices for molecular biology is essential to the proper performance of the qPCR or any laboratory experiment. Attention should be taken to the procedures particular to the molecular diagnostics procedures. Due to the high sensitivity of **Logix Smart SARS-CoV-2 DS** 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 pipette tips with filters that are DNase and RNase free.
- 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 SARS-CoV-2 DS** 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, HANDLING, TRANSPORT, AND STORAGE

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

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

CDC - <https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html>

WHO - <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>

a) **Saliva**

Collect 1 – 5 mL of saliva into a sterile or nuclease free, leak-proof, screwcap container. Do Not use preservatives. No food or drink should be consumed within 30 minutes of sample collection.

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**

Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below. 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 with ice packs 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 with enough ice packs to keep it cold throughout transit. After the collection of the sample and transfer to the clinical lab, the sample will receive an entry into the laboratory system.

7 PROCEDURE

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

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

7.1 Sample Processing

The **Logix Smart SARS-CoV-2 DS** test kit requires no sample extraction, only a brief sample processing procedure.

1. Vortex the saliva sample for 1 minute to help reduce the viscosity of the sample.
2. Add 2.5 µL of sample processing solution to a microcentrifuge tube, strip tube, or plate along with 50 µL of the vortexed saliva and cover.
3. Vortex for 1 minute, and briefly centrifuge.
4. Heat the sample at 95 °C for 5 minutes in a heat block or thermocycler to lyse the cells, releasing the nucleic acids, and inactivating the proteinase K.
5. Allow samples to cool to room temperature before plating.

7.2 Logix Smart SARS-CoV-2 DS Reagent Setup

- When preparing reagents, clean all working surfaces with fresh 10% bleach solution followed by molecular grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.
- All **Logix Smart SARS-CoV-2 DS** Master Mix, Positive Control (PC), nuclease-free water (used as a no template control or NTC), and sample tubes should be vortexed for approximately 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 10 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 10 µL of the patient sample (after heat treatment) or 10 µL of a control (**NTC** and **PC**) to the appropriate well(s). At least one positive control and one NTC 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 the real-time PCR instrument in the correct orientation and start the run.

7.4 qPCR Instrument Setup for the CoDx Box

Contact the Laboratory 801-438-1036 ext. 04 or at www.codiagnosics.com/contact/ for the template file for download for use with the CoDx Box. The template file comes pre-programmed with the PCR instrument setup described in the section below.

7.5 qPCR Instrument Setup

- 7.5.1 Define the following settings:

Reaction Volume	20 µL
Ramp Rate	Default
Passive Reference	None

- 7.5.2 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	45	95°C	3 seconds
		55°C	32 seconds

7.5.3 Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
SARS-CoV-2 <i>(RdRp & E gene)</i>	SARS-CoV-2	FAM™	BHQ® - 1
Human RNaseP specific DNA (IPC)	RNaseP	CAL Flour® Red 610	BHQ® - 2

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

8 DATA ANALYSIS

Verification and validation studies performed for **Logix Smart™ SARS-CoV-2 DS** (Catalog number COVDS-K-003 or COVDS-K-003-01) 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. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

8.1 Analysis Settings

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

- Check the box to **Auto Set Threshold**.
- “Method” should be set to **Dynamic**.
- “Threshold Level” should be set to **0.100**.
- “Threshold Start” should be set to **1.00**.
- “Ignore Cycles Before” should be set to 5.
- “Exclusion” should be set to **Extensive**.
- “Fluorescence Cutoff Level” should be set to **5.0%**.
- “Initial Y-Axis Scale” should be set to **Linear**.
- “Auto Generate Analysis” should be checked.

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

8.2 Positive Controls

Highlight the positive control reaction well. Each positive control should show an amplification curve for SARS-CoV-2 in the FAM channel, and amplification of the internal positive control for RNaseP (IPC) in the CF610 channel. A positive amplification curve looks like the purple curve in Figure 8.2 and should have a Cq value below 40 cycles.

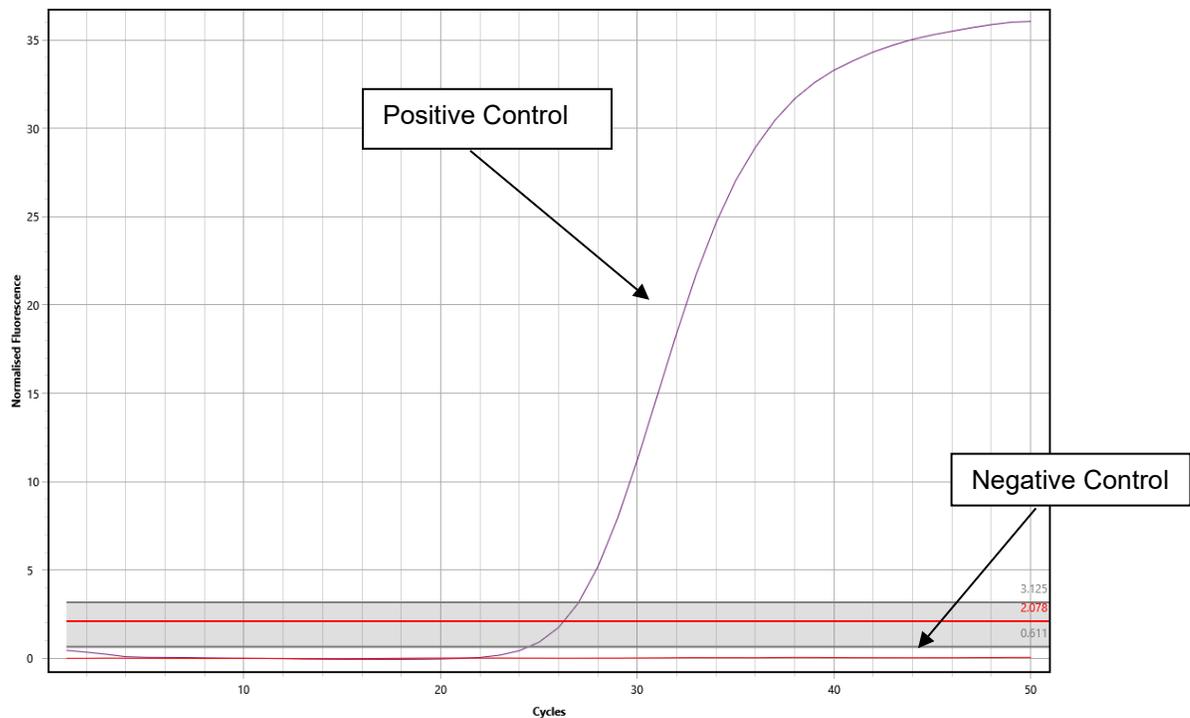


Figure 8.2: Positive Control (PC) and No Template Control (NTC) signals for Logix Smart SARS-CoV-2 DS

8.3 Negative Control

Next highlight the negative control. The results of the negative control should show no amplification, specifically with a Cq value less than 40. An example of no amplification can be seen in Figure 8.2, as the red line, which is below the threshold area. The threshold area is the grey band with the red line.

8.4 The Validity of the Diagnostic Test Runs

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

After the run completes, the results can be interpreted by evaluating the Ct (Cq) values. The process for evaluating results are as follows:

- Verify that the positive control (PC) showed amplification for SARS-CoV-2, and RNaseP in the appropriate channels. There should be amplification in every channel for the PC before cycle 40.
- Verify that the no template control (NTC) does not show amplification of SARS-CoV-2, and RNaseP in the appropriate channel. There should be NO amplification in any of the channels before cycle 40.
- Verify that the internal positive control (IPC) for RNaseP shows amplification in the appropriate channel. Every sample should show a positive result for RNaseP (IPC) before cycle 40.

The following control conditions must be met:

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

If controls pass, interpret the sample results.

If the controls are not valid, the patient results cannot be interpreted. A new plate with the same layout should be rerun and the controls should be re-evaluated. If it fails again, the kit should be quarantined in an appropriate location and a new kit should be opened and tested. This should be done after internal investigation for potential deviations.

8.5 Interpretation of Results

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

- Positive
- Negative
- Invalid

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

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

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

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

Table 8.1 Interpretation of Results for SARS-COV-2 detection with Logix Smart SARS-CoV-2 DS

	Sample Result		Logix Smart™ SARS-CoV-2 DS Positive Control	No Template Control (NTC) (Master Mix + Water)	Interpretation of Results*
	SARS-CoV-2 (FAM™)	RNaseP (IPC) (CF@610)			
Instrument Reading	+	+	+	-	SARS-CoV-2 RNA +
	-	+	+	-	SARS-CoV-2 RNA -
	Any Result (+/-)	-	+	-	INVALID: See Troubleshooting
	Any Result (+/-)	+	-	-	INVALID: See Troubleshooting
	Any Result (+/-)	+	+	+	INVALID: See Troubleshooting
Amplification before 40 cycles is considered a positive reading (+). Amplification after 40 cycles is considered a negative reading (-). When possible, always check that the medical history and/or symptoms match the result before initiating treatment.					

* The following statement will also appear together with the Interpretation of Results: *[The Logix Smart SARS-CoV-2 DS Test has been validated by Co-Diagnostics, but the US FDA's independent review of this validation is pending.]*

9 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Logix Smart™ SARS-CoV-2 DS** 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/](https://www.codiagnostics.com/contact/feedback/)

9.1 Stability

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

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

9.2 User Errors

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

The user needs to have some molecular biology experience and be familiar with the proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection. Pipette tips must be

replaced after every pipetting. Gloves must be replaced often. Equipment must have calibration up to date for the pipettes and thermocyclers, when applicable.

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

9.3 Invalid Results

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

Without further evidence, the run should be considered invalid and the user should re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the patient samples may need to be treated and tested again. If failure of the positive control happens a third time after re-treating and re-amplification, open a new **Logix Smart SARS-CoV-2 DS 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.codiagnosics.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,
- the sample preparation was performed incorrectly,

If the internal positive control (IPC) shows no amplification, re-testing should be performed. If the IPC fails again, then samples should be re-processed by diluting the saliva sample 1:2 with 10% TE Buffer and re-run on the thermocycler. If it fails a third time an investigation should be conducted to identify possible causes for error.

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

Note: Positive amplification in the SARS-CoV-2 channel may indicate 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 heat-treated sample, the amount of which is governed by the quality of the patient sample and the sample treatment performed.

In the investigation the two possible scenarios should be evaluated:

- The positive result for SARS-CoV-2 (FAM) channel is a true positive while the IPC is negative due to the lack of human RNaseP gene in the sample (absence of human cells in the sample).

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

Failure of any of the controls may indicate that the sample heat-treatment or sample collection have failed.

If the IPC persists to be negative with negative SARS-CoV-2 channel the result should be reported as INVALID with “NEW SAMPLE COLLECTION NEEDED” request.

If the cause for an error is unclear, contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or contact us at www.codiagnostics.com/contact/.

9.3.3 No Template Control showing amplification

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

The results should be interpreted as invalid and re-testing by re-amplification should be performed. If the NTC fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the patient samples may need to be heat treated and tested again. If failure of the NTC, after re-treating and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, the run should be interpreted as invalid. Please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 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 assay is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests. Laboratories should include a statement in test reports to healthcare providers that the test has been validated by Co-Diagnostics, but the US FDA’s independent review of this validation is pending.
- The use of this product is to be limited to trained and instructed personnel in real-time PCR techniques and IVD procedures.
- Good laboratory practices are essential for the proper performance of this assay. It is also recommended that upon receipt of reagents that a test run be performed to check the performance of the reagents before testing on patient samples.
- Appropriate specimen collection, transport, storage, and processing procedures are required for optimal results.
- Do not use the **Logix Smart SARS-CoV-2 DS** kit components directly on the specimens collected. Perform an appropriate sample preparation (addition of Proteinase K and heat treatment) before using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the COVID-19 genome covered by this test kit may fail to detect the presence of the pathogens.
- As with any diagnostic test, results of the **Logix Smart SARS-CoV-2 DS** kit are to be interpreted with consideration of all clinical and laboratory findings.

11 ANALYTICAL EVALUATION

The analytical evaluation of performance was performed with contrived samples produced by spiking in NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2), Isolate USA-WA1/2020 (Cat. No. NATSARS(COV2)-ST, ZeptoMetrix) in confirmed negative saliva samples.

11.1 Limit of Detection (LoD) – Analytical Sensitivity

Limit of Detection (LoD) is the lowest concentration of analyte that is detected at a rate of no less than 95%. The experiment was performed using technical samples prepared by spiking in NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2), Isolate USA-WA1/2020 (Cat. No. NATSARS(COV2)-ST, ZeptoMetrix) into confirmed negative saliva and processed using the Logix Smart SARS-CoV-2 DS protocol. The method of choice to establish the Limit of Detection includes the treatment with the CoDx Sample Processing Solution (Cat. No. SPS-DSR01, Co-Diagnostics) and run on CoDx Box Cycler (Cat. No. CDX-MC4, Co-Diagnostics). The LoD was evaluated for each thermocycler individually with evaluation of equivalence to the method of choice. The following Proteinase K reagent solutions were used in the study: CoDx Sample Processing Solution and MagMAX Viral/Pathogen Proteinase K. The following thermocyclers were used in the study: CoDx Box Cycler, MIC qPCR Cycler, QuantStudio 5, CFX 96 Touch, and ABI 7500 Fast. Each tentative LoD concentration was confirmed by 20 replicates. The **Table 11.1** shows the results.

Table 11.1 Confirmation of the LoD with 20 replicates

Thermocycler	Sample treatment method	Concentration (copies/μL)	Call	Detection Rate
CoDx Box Cycler	CoDx Sample Processing Solution	3.0	19/20	95%
MIC qPCR Cycler	CoDx Sample Processing Solution	1.7	19/20	95%
QuantStudio 5	CoDx Sample Processing Solution	3.5	19/20	95%
CFX 96 Touch	CoDx Sample Processing Solution	5.0	19/20	95%
ABI 7500 Fast Dx	CoDx Sample Processing Solution	2.0	20/20	100%
ABI 7500 Fast Dx	MagMAX™ Viral/Pathogen Proteinase K	1.0	20/20	100%

11.2 Inclusivity (Analytical Sensitivity):

1.2.1 *In silico* inclusivity

An alignment was performed with the oligonucleotide CoPrimer sequences of the genes RdRp and E CoPrimers with publicly available nucleic acid sequences for SARS-CoV-2. 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 27-Jan-2020 with posterior queries performed monthly. Sequences were obtained from <https://github.com/nextstrain/ncov/blob/master/data/metadata.tsv>

Each marker in Logix Smart SARS-CoV-2 is expected to detect strains with a single mismatch without difficulty. At 2 mismatches, each marker in Logix Smart SARS-CoV-2 DS is expected to detect with significant Cq delay. Events of 3+ mismatches are expected to lead to no detection by that marker. To maintain 99%+ expected sensitivity for

both markers, 99%+ of the sampled sequences should maintain less than three mismatches on either marker. To maintain 99%+ expected sensitivity for either marker, 99%+ of the sampled sequences should maintain <3 mismatches on both markers. The most recent monitoring performed in Jan/2021 results are shown in **Table 11.2**.

Table 11.2 In Silico Inclusivity for Logix Smart SARS-CoV-2 DS

	100 % homology (No mismatches)	98% homology (1 mismatch)	95% homology (2 mismatches)
RdRp conservation	99.01%	0.99%	0%
E-gene conservation	99.72%	0.28%	0%

Recently reported mutations with new variants in the UK (known as 20I/501Y.V1, VOC 202012/01, or B.1.1.7), in South Africa (known as 20H/501Y.V2 or B.1.351), and in Brazil (known as P.1 or 20J/501Y.V3) have been investigated. None of the new variants have been reported with mutations outside of the S gene. In the FDA Letter to Healthcare Providers (U.S. FDA, 2021) the FDA highlights that the recent variants targeting gene S should not impact real-time PCR tests targeting other genes. Therefore, as the Logix Smart SARS-CoV-2 targets the genes RdRp and E, none of the recent new variants are expected to show impact in the sensitivity or specificity of the test kit.

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

In Silico Analysis BLASTn analysis queries of the SARS-CoV-2 CoPrimers were performed against public domain nucleotide sequences. The database search parameters were as follows: The database search parameters were as follows: 1) The nucleotide collection consisted of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excluded EST, STS, GSS, WGS, TSA, patent sequences, phase 0, 1, and 2 HTGS sequences, and sequences longer than 100Mb; 2) The database was non-redundant. Identical sequences were merged into one entry, while preserving the accession, GI, title, and taxonomy information for each entry; 3) The search parameters automatically adjusted for short input sequences and the expect threshold is 1000; 4) The match and mismatch scores were 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment was 5 and 2 respectively; 7) BLASTn was run individually for every organism listed in **Table 11.3**.

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

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

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

Table 11.3 Relevant Microorganisms Investigated for cross-reactivity

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	<i>Corynebacterium diphtheriae</i>
Human coronavirus NL63	Influenza A & B	<i>Legionella non-pneumophila</i>
SARS-coronavirus	Enterovirus	<i>Bacillus anthracis</i> (Anthrax)
MERS-coronavirus	Respiratory syncytial virus	<i>Moraxella catarrhalis</i>
	Rhinovirus	<i>Neisseria elongata</i>
	<i>Chlamydia pneumoniae</i>	<i>Neisseria meningitides</i>
	<i>Haemophilus Influenza</i>	Leptospirosis
	<i>Legionella pneumophila</i>	<i>Chlamydia psittaci</i>
	<i>Mycobacterium tuberculosis</i>	<i>Coxiella burnetii</i> (Q-Fever)
	<i>Streptococcus pneumoniae</i>	<i>Staphylococcus aureus</i>
	<i>Streptococcus pyogenes</i>	
	<i>Bordetella pertussis</i>	
	<i>Mycoplasma pneumoniae</i>	
	<i>Pneumocystis jirovecii</i> (PJP)	
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract	
	<i>Candida albicans</i>	
	<i>Pseudomonas aeruginosa</i>	
	<i>Staphylococcus epidermidis</i>	
	<i>Staphylococcus salivarius</i>	

11.4 Wet-test Exclusivity

A wet test was performed as part of the exclusivity investigation to confirm that the **Logix Smart SARS-CoV-2 DS kit** does not cross react with non-target organisms. The test was performed by spiking negative saliva with non-target organisms reference material. The materials that were already extracted were spiked post extraction. Non-target organisms were spiked in at a final concentration of 1e4 copies/rxn (1e3 copies/μL (1e6 copies/mL)) and run in duplicate.

The data generated from the specificity-exclusivity runs are summarized below in **Table 11.4**. There was no amplification of non-target organism, with the exception of SARS-CoV-1 (2003) which have shown potential for cross-reactivity on in silico analysis.

Table 11.4 Logix Smart SARS-CoV-2 DS Exclusivity Testing

Sample	Strain/Isolate	SARS-CoV-2 Cq
Human coronavirus OC43	Betacoronavirus 1 OC43	-
Human coronavirus HKU1	Human coronavirus HKU1	-
Human coronavirus NL63	Human coronavirus NL63	-
SARS-coronavirus (2003)	HKU-39849	34.37
MERS-coronavirus	Betacoronavirus England-1	-
Human Metapneumovirus (hMPV)	TN/91-316	-
Parainfluenza virus 3	C 243	-
Enterovirus (e.g., EV68)	F02-3607 Corn	-
Respiratory syncytial virus	Long	-
Rhinovirus	1059	-
<i>Chlamydia pneumoniae</i>	TW-183	-
<i>Haemophilus influenzae</i>	Type B	-
<i>Legionella pneumophila</i>	Philadelphia 1	-
<i>Mycobacterium tuberculosis</i>	H37Rv	-
<i>Streptococcus pneumoniae</i>	GA17545	-
<i>Streptococcus pyogenes</i>	MGAS1882, serotype M59	-
<i>Bordetella pertussis</i>	F	-
<i>Mycoplasma pneumoniae</i>	FH of Eaton Agent	-
Pneumocystis jirovecii (PJP)	Rat prototype	-
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract	Not available	-
<i>Candida albicans</i>	NIH 3172	-
<i>Pseudomonas aeruginosa</i>	R. Hugh 813	-
<i>Staphylococcus epidermidis</i>	PCI 1200	-
<i>Streptococcus salivarius</i>	DSM 13084	-

Note: "-" means no amplification

11.5 Clinical Evaluation

Samples were procured and tested by Nomi Health Laboratory Services (Salt Lake City, UT) as the third-party clinical laboratory service provider. The clinical evaluation was conducted by testing twenty-five remnant SARS-CoV-2 positive saliva specimens with addition of five 1:10 dilutions of high positive samples, according to guidance from the American Society for Microbiology (Mitchell, et al., 2020). Additionally, thirty fresh SARS-CoV-2 negative paired saliva and anterior nasal swabs were procured by Nomi Health in Dec/2020. The remnant positive samples were procured in October/2020, aliquoted, tested, and adequately maintained at -70°C. The comparator assay was the laboratory developed test validated internally by Nomi Health.

The 30 known positive remnant saliva and the fresh 30 negative specimens were treated with the CoDx Sample Processing Solution (Cat. no. SPS-DSR01) (Proteinase K solution) to expose the genetic material, then processed with Logix Smart SARS-CoV-2 DS (COVDS-K-003) according to instructions for use and run on ABI 7500 Fast Dx.

The 30 fresh negative paired anterior nasal swab and saliva specimen were processed according to the laboratory internal procedures with automated extraction with KingFisher and run on the ABI 7500 Fast Dx.

Of the 30 remnant positive saliva samples, 29 had a positive call with Logix Smart SARS-CoV-2 DS and were in agreement with the sample call. Of the 30 negative paired saliva and anterior nasal swab specimens, all 30 pairs were negative with the comparator assay, the 30 negative saliva paired samples were processed with the Logix Smart SARS-CoV-2 DS kit and found in 100% agreement with the comparator assay. The results are summarized in **Table 11.5**.

Table 11.5 Summary of Results for Clinical Evaluation of Logix Smart SARS-CoV-2 DS

		Logix Smart SARS-CoV-2 DS (Saliva)	
		Positive	Negative
Comparator LDT (Paired saliva and anterior nasal swab)	Positive	29	1
	Negative	0	30
Positive Agreement (PPA)		96.7% (29/30)	
Negative Agreement (NPA)		100% (30/30)	

12 MANUFACTURER AND AUTHORIZED REPRESENTATIVE


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Rx only

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14 LEGEND OF PACKAGE SYMBOLS

	<i>In vitro</i> diagnostic medical device		Contains sufficient for 100 or 2,500 reactions
	Catalog number		Protect from light
	Batch Code		Temperature limit
	Cap color		Consult Instructions for Use
	Content/Volume		Non-Sterile product – Do not sterilize
	Number		Manufacturer
	Use-by-date		