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Logix Smart™ SARS-CoV-2 DS RUO

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

REF

COVDS-R-003 (without PK) /
COVDS-R-004 (with PK)

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

RUO



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1 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

2 INTENDED USE

The **Logix Smart™ SARS-CoV-2 DS RUO** is a real-time multiplex RT-PCR test, using a proprietary primer technology called CoPrimers™, intended for the detection of nucleic acid from SARS-CoV-2, targeting the *RdRp* and *E* genes, in saliva. **For research use only. Not for use in diagnostic procedures.**

Results are for the identification of SARS-CoV-2 RNA. Positive results are indicative of the presence of SARS-CoV-2 RNA.

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

3 PRODUCT DESCRIPTION

The **Logix Smart SARS-CoV-2 DS RUO** is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test utilizing 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. This test uses a sample processing technique that eliminates the use of the nucleic acid extraction/purification process.

4 RUO COMPONENTS

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

See Table 1 for components included in the Logix Smart SARS-CoV-2 DS without PK and Table 2 for components include in the Logix Smart SARS-CoV-2 DS with PK.

Table 1

Components Included in the Logix Smart SARS-CoV-2 DS without PK (COVDS-R-003)*

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	1×1000 µL (100 reactions) or 1×25000 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	1×1000 µL (100 reactions) or 1×25000 µL (2,500 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1×1000 µL (100 reactions) or 1×25000 µL (2,500 reactions)

*Components are not sold separately.

Table 2

Components of Logix Smart SARS-CoV-2 DS with PK (COVDS-K-004)*

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Brown	Logix Smart SARS-CoV-2 DS Master Mix	MM	COVDS-MM-004	Proprietary blend of SARS-CoV-2 CoPrimers and PCR reagents	1×1000 µL (100 reactions) or 1×25000 µL (2,500 reactions)
Red	Logix Smart SARS-CoV-2 DS Positive Control	PC	COVDS-PC-004	Proprietary blend of SARS-CoV-2 and Human RNaseP synthetic templates	1×1000 µL (100 reactions) or 1×25000 µL (2,500 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1×1000 µL (100 reactions) or 1×25000 µL (2,500 reactions)
Purple	Sample processing Solution	SPS	SPS-DSR01	Solution of Proteinase K	1×300 µL (100 reactions) or 1×7500 µL (2,500 reactions)

*Components are not sold separately.

5 STORAGE

Note the following during storage and handling:

- If one or more of the components are not frozen upon receipt or are compromised during shipment, contact your distributor. The **Logix Smart SARS-CoV-2 DS RUO** test is shipped on dry ice and the components should arrive frozen.
- Store all components immediately at -40°C to -16°C to prevent degradation of reagents.
- Always work with each **Logix Smart SARS-CoV-2 DS RUO** component on ice. Make aliquots, if necessary, to avoid 10 or more 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 RUO** test remains frozen.
- Store the Sample Processing Solution, containing Proteinase K, at -40°C to -16°C. It does not freeze at this temperature range but maintains its liquid state.
- Stability data for the product is currently being collected, results will be published, and new Instructions for Use updated will reflect the stability conditions.

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 lab, the sample will receive an entry into the laboratory system.

6 MATERIAL REQUIRED (NOT INCLUDED)

Consumables required but not provided include the following:

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

The sample processing reagents compatible for use with the Logix Smart SARS-CoV-2 DS RUO are displayed in Table 3. Sample Processing Solution (Co-Diagnostics, Inc., SPS-DSR01) is only provided with the COVDS-K-004 test.

Table 3

Sample Processing Reagents Compatible with but Not Included with the Test

Sample Treatment Reagent	Manufacturer	Catalog #	Volume Added to Sample
Sample Processing Solution	Co-Diagnostics	SPS-DSR01	2.5 µL
MagMAX™ Viral/Pathogen Proteinase K	Thermo Fisher Scientific	A42363	2.5 µL

Equipment that is required but not provided includes the following:

- Several micropipettes capable of pipetting volumes from 5 µL to 1000 µL
- A cold block or ice
- A vortex and centrifuge
- A Class II Biosafety cabinet, ideally in a BSL-2 containment facility, for sample processing
- A heat block capable of reaching 95°C
- A PCR workstation, for master mix plating and setup
- A thermocycler (See Table 4)

Table 4

Thermocyclers Compatible with but Not Included with the Test

Thermocycler Machine	Catalog Number	Manufacturer	Number of wells
CoDx Box Cycler	CODX-BOX-001	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

7 BACKGROUND INFORMATION

Coronavirus (2019-nCoV) was initially reported to WHO on December 31, 2019, as an outbreak in Wuhan City, Hubei Province, China of pneumonia of unknown etiology. Thousands of human infections were confirmed in that region, resulting in exported cases worldwide. Cases involved severe illness to death. Based on this worldwide spread of the

contagion, countries and the WHO called for diagnostic testing to identify, detect and diagnose coronavirus (2019-nCoV) and its emerging variants.

8 ACCESSORIES (NOT INCLUDED)

8.1 Thermocycler

Co-Diagnostics, Inc. can either directly or through reagent rental programs provide the CoDx Box™ thermocycler machines (manufactured for Co-Diagnostics, Inc. by Bio Molecular Systems). Logix Smart SARS-CoV-2 DS RUO can also be used in other real-time PCR systems as long as the parameters to run the test are set as established in the Logix Smart SARS-CoV-2 DS RUO. The CoDx Box thermocycler is recommended due to its ease of use, small size, durability, and fast report generation. The CoDx Box thermocycler software was developed by Bio Molecular Systems solely for Co-Diagnostics, Inc., and it has been verified for use with Co-Diagnostics, Inc. real-time PCR products, simplifying result interpretation. The CoDx Box thermocycler reads fluorescence in real-time, generated from the PCR reagents loaded into CoDx Box PCR reaction tubes, amplifies the virus RNA by thermal cycling using magnetic induction, and displays output data through the integrated software. The CoDx Box thermocycler is available with 48 reaction wells and 4 channels. Other Co-Diagnostics, Inc. real-time PCR products also utilize this CoDx Box thermocycler. The Microsoft Surface™ Pro 4 System (MSPRO-4) is available for use with CoDx Box software in a windows-based operation system. The output device used with the CoDx Box thermocycler can be a printer or external computer. Alternately, the results can be manually recorded. The method of reporting is left to the discretion of the user.

8.2 Extraction Kit

Logix Smart SARS-CoV-2 DS RUO requires no sample extraction, only a brief sample processing procedure. If sample extraction is performed, the quality of the extraction of the RNA from the samples is essential for the performance of Logix Smart SARS-CoV-2 DS RUO. The extraction protocol to be followed should be performed following manufacturer's instructions or an internally validated protocol.

9 WARNINGS AND PRECAUTIONS



WARNING!

Before performing any testing or running any 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. Particular attention should be taken to the molecular diagnostics procedures. Due to the high sensitivity of **Logix Smart SARS-CoV-2 DS RUO** and the qPCR technology, care

should be taken while handling samples and materials while performing the assay to keep reagents and amplification mixtures free of contamination. Users should do the following:

- Use sterile pipette tips with filters.
- Use standard precautions when handling any samples, as they may contain infectious agents.
- Store and process positive materials (specimens, positive controls, and amplicons) separately from other reagents.
- Always use nuclease-free water, provided with this RUO.
- 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://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 <http://co-dx.com/resources/instructions-for-use/>

10 SAMPLE INFORMATION

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 samples, 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>

10.1 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. Avoid repeated freezing and thawing of a specimen. 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).

10.2 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.

Samples 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).

When collecting samples, collect 2-3 mL of saliva into a sterile, leak-proof, screwcap container. No food or drink should be consumed within 30 minutes of sample collection.

For specific instructions on the handling of specimens associated with 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).

The **Logix Smart SARS-CoV-2 DS RUO** test requires no sample extraction, only a brief sample processing procedure. To process a sample, perform the following steps:

- 10.2.1 Vortex the saliva sample for 1 minute to help reduce the viscosity of the sample.
- 10.2.2 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.
- 10.2.3 Vortex for 1 minute, and briefly centrifuge.
- 10.2.4 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.
- 10.2.5 Allow samples to cool to room temperature before plating.

11 PROCEDURE

11.1 Real Time RT-PCR Setup

11.1.1 Set Up the Reagent

Perform the steps below to set up the reagent.

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

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

11.1.1.3 Vortex all Logix Smart SARS-CoV-2 DS MM, PC, nuclease-free water (used as a no template control or NTC), and all sample tubes for 3 seconds.

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

11.1.2 Set Up the Reaction

Perform the steps below to set up the reaction.

11.1.2.1 Collect enough reaction wells for each of the following:

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

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

Positive control 1
NTC 1
Samples 5
Total wells needed 7

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 NTC.

Change pipette tips between samples and change pipette tips after pipetting each component.

Pipet the PC last, if possible, to avoid contamination events.

11.1.2.2 Pipet 10 µL of MM into each well collected.

11.1.2.3 Pipet 10 µL of the sample or 10 µL of NTC control to the appropriate wells (in addition to the 10 µL of MM already in the well).

Note: Ensure to include at least one NTC control in each run and that enough space remains for at least one PC.

- 11.1.2.4 Pipet 10 µL of PC into the appropriate well.
- 11.1.2.5 Seal the reaction plate with an optical adhesive film or seal each reaction tube with its appropriate lid.
- 11.1.2.6 Place the plate or tubes into the real-time PCR instrument in the correct orientation and start the run.

11.2 Thermocycler Setup

In case of questions or assistance handling or using Logix Smart SARS-CoV-2 DS RUO, contact the Co-Diagnostics, Inc. Laboratory (USA) by calling +1 801-438-1036 ext. 3, or email Customer Support support@co-dx.com, or visit our website at www.co-dx.com/contact/.

11.2.1 qPCR Instrument Setup for the CoDx Box

When using the CoDx Box Cycler (CODX-BOX-001), contact customer support by email support@co-dx.com, or visit our website at www.co-dx.com/contact/ for download of the template file for a quick setup of the CoDx Box Cycler. The template file comes pre-programmed with the PCR instrument setup described in the section below.

When using other thermocycler than the CoDx Box (CODX-BOX-001), please, follow the instrument’s manual of instructions to manually enter the PCR instrument setup parameters described below.

11.2.2 qPCR Instrument Setup

- 11.2.2.1 Define the settings as displayed in Table 5.

Table 5

qPCR Instrument Setup

Item	Setting
Reaction Volume	20 µL
Ramp Rate	Default
Passive Reference	None

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

Table 6

Program for PCR Instrument with Cycling Conditions

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

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

Table 7

Fluorescence Detectors (Dyes)

Target	Detector Name	Reporter	Quencher
SARS-CoV-2 (RdRp & E gene)	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.

12 DATA ANALYSIS

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Verification and validation studies performed for **Logix Smart™ SARS-CoV-2 DS** (Catalog number COVDS-R-003 / COVDS-R-004) 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 sample results. If the controls are not valid, the sample results cannot be interpreted.

Note the following regarding the validity of the test runs:

- All test controls should be examined prior to interpretation of sample results. If the controls are not valid, the sample 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.

Ensure the control conditions displayed in Table 8 are met.

Table 8

Required Control Conditions

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 sample 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 SARS-CoV-2 DS RUO Test should be quarantined in an appropriate location and a new package should be opened and tested. This should be done after internal investigation for potential deviations.

12.1 Interpretation of Results

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.

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 sample processing 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 from the same source 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 the Troubleshooting section for more information.

Translate the interpretation of results with Ct values to Table 9.

Table 9

Interpretation of Results for SARS-COV-2 with Logix Smart™ SARS-CoV-2 DS (COVDS-R-003 / COVDS-R-004)

	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 Detected
	-	+	+	-	SARS-CoV-2 RNA Not Detected
	Any Result (+/-)	-	+	-	INVALID: See Troubleshooting
	Any Result (+/-)	+	-	-	INVALID: See Troubleshooting
	Any Result (+/-)	+	+	+	INVALID: See Troubleshooting

Amplification before 40 cycles is considered a positive analyte reading (+). Amplification after 40 cycles is considered a negative analyte reading (-).

13 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Logix Smart™ SARS-CoV-2 DS** RUO test even if the recommended step for troubleshooting solves an issue. To give feedback please email Customer Support Team at support@co-dx.com.

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

Always use the most recent version of this document. For updates consult our website at co-dx.com/resources/instructions-for-use/.

In case of questions handling or using this product, please contact Co-Diagnostics Inc. Customer Support by calling (USA) +1 801-438-1036, ext. 02, or email support@co-dx.com.



Co-Diagnostics, Inc. (CoDx) products' information can be found at <https://co-dx.com/products/diagnostic-solutions/>.

13.1 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.

13.2 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 needs to 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 up-to-date calibration for the pipettes and thermocyclers, when applicable.

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

13.3 Invalid Results

13.3.1 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 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 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 -16°C for an extended period)
- Use of expired reagents
- 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 original 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.

13.3.2 RNaseP (IPC) not amplifying in samples.

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

- Not enough nuclear material was in the original 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 original 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 a NEW SAMPLE COLLECTION NEEDED request.

13.3.3 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 original samples may need to be processed 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 the cause for an error is unclear, contact Co-Diagnostics Inc. Customer Support by calling (USA) +1 801-438-1036 ext. 2 or by email at support@co-dx.com.

14 REFERENCES

- CDC. (2009). *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*. Retrieved from CDC Laboratories: <https://www.cdc.gov/labs/BMBL.html>
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15 LEGEND OF PACKAGE SYMBOLS

See Table 10 for a legend of package symbols.

Table 10

Legend of Package Symbols

Icon	Description
	Catalog number
	Batch code
	Cap color
	Component
	Content/volume
	Number
	Use-by-date
	Contains sufficient for 100, 250 or 5,000 reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Non-sterile product – do not sterilize
	Manufacturer
	Research Use Only