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CO-DIAGNOSTICS INC.

Logix Smart™ SARS-CoV-2 DS

For *in vitro* diagnostic use
For Professional Use Only

REF **COVDS-K-003 (without PK) / COVDS-K-004 (with PK)**

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IVD

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

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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 from individuals suspected of COVID-19. Testing is limited to clinical laboratories environment and operations.

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. Many laboratories are required to report all positive results to the appropriate public health authorities, laboratories must follow local regulations for reporting requirements.

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.

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 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 with a brief treatment with Proteinase K. 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 ºC 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. , 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.

Table 2.1 Components included in the Logix Smart SARS-CoV-2 DS without PK (COVDS-K-003) Kit.*

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)

*Kit components are not sold separately.

Table 2.2 Components of Logix Smart SARS-CoV-2 DS with PK (COVDS-K-004) Kit*

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	1x1000 µL (100 reactions) or 1x25000 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	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)
Purple	Sample processing Solution	SPS	SPS-DSR01	Solution of Proteinase K	1x300 µL (100 reactions) or 1x7500 µL (2,500 reactions)

*Kit components are not sold separately.

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.
- Note that the Sample Processing Solution containing Proteinase K, must also be stored below -20°C, however, it does not freeze at -20°C, maintaining its liquid state.
- 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 sample processing reagents validated for use with the Logix Smart SARS-CoV-2 DS are shown in **Table 4.1** below. Only Sample Processing Solution (Co-Diagnostics, Inc., SPS-DSR01) is provided in the kit COVDS-K-004.

Table 4.1 Sample Processing Reagents Validated 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

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	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

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 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 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 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 2-3 mL into a sterile, leak-proof, screwcap container. 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 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, etc.).

In case of questions or assistance handling or using **Logix Smart SARS-CoV-2 DS**, contact the Co-Diagnostics, Inc. Laboratory (USA) by calling +1 801-438-1036 ext. 4, or email Customer Support support@codiagnostics.com, or visit our website at www.codiagnostics.com/contact/.

7.1 Sample Processing

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

Step 1: Vortex the saliva sample for 1 minute to help reduce the viscosity of the sample.
Step 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.
Step 4: Vortex for 1 minute, and briefly centrifuge.
Step 5: 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.
Step 6: 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 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

When using the CoDx Box Cycler (CODX-BOX-001), contact customer support by email support@codiagnostics.com, or visit our website at www.codiagnostics.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.

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 (*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.

8 DATA ANALYSIS

Verification and validation studies performed for **Logix Smart™ SARS-CoV-2 DS** (Catalog number COVDS-K-003 / COVDS-K-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 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

Identify or 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.

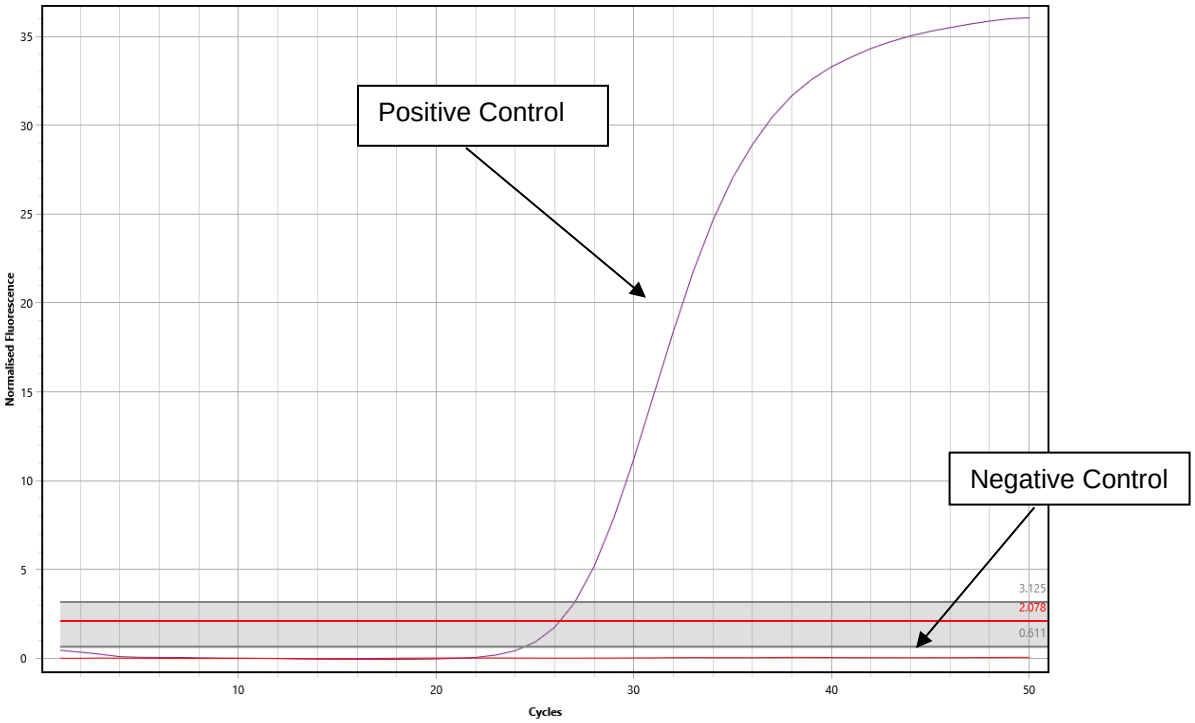

Figure 8.2: Positive Control (PC) and No Template Control (NTC) signals for Logix Smart SARS-CoV-2 DS (COVDS-K-003 / COVDS-K-004)

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 with Logix Smart SARS-CoV-2 DS (COVDS-K-003 / COVDS-K-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 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.

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 step for troubleshooting solves an issue. To give feedback please email Customer Support Team at support@codiagnostics.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 codiagnostics.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 extension 4, or email support@codiagnostics.com.

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

9.1 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

9.2 Invalid Results

9.2.1 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.

9.2.2 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.

9.2.3 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 the cause for an error is unclear, contact Co-Diagnostics Inc. customer support by calling (USA) +1 801-438-1036 ext. 4 or by email at support@codiagnostics.com.

10 LIMITATIONS

- Strict compliance with this document is required for optimal results. Please, always use the most recent version of this document. This most recent version 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 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 Precision (Repeatability)

The precision was performed over 5 days with 3 shifts run per day. Samples were prepared using the reference materials NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2), Isolate USA-WA1/2020 (ZeptoMetrix, catalog number NATSARS(COV2)-ST) for the low concentration, and SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI Resources, catalog number NR-52287) for the medium and high concentrations. Reference material was spiked into saliva and then treated according to the procedure described in 7.1.1. The concentrations used are identified as [High] = 100,000 copies/µL, [Medium] = 300 copies/µL, and [Low] = 9 copies/µL. The average Cq's for each run should be within 2 cycles of the Cq average that day.

Table 11.1 p-Value in ANOVA for Precision Study of Logix Smart SARS-CoV-2 DS

[SARS-CoV-2]	p-Value (Days)
COVID [Low]	8.18E-9
COVID [Medium]	1.60E-8
COVID [High]	6.07E-11

Table 11.2 Combined Precision Results for Logix Smart SARS-CoV-2 DS

	Cq Average	SD	Call Rate	CV%	Detection Rate (%)
COVID [Low]	35.12	1.05	75/75	3.00	100%
COVID [Medium]	30.85	1.26	75/75	4.07	100%
COVID [High]	22.06	1.42	75/75	6.43	100%

11.2 Analytical Sensitivity - Limit of Detection (LoD)

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 11.3** shows the results.

Table 11.3 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	CoDx Sample Processing Solution	**5.0**	19/20	95%
7500 Fast Dx	CoDx Sample Processing Solution	**2.0**	20/20	100%
7500 Fast Dx	MagMAX™ Viral/Pathogen Proteinase K	**1.0**	20/20	100%

11.3 Analytical Specificity - Inclusivity

11.3.1 Inclusivity performed by *in silico* analysis.

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 phylogenic 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://academic.oup.com/bioinformatics/article/34/23/4121/5001388 7/6/2021.

Each marker in Logix Smart SARS-CoV-2 DS is expected to detect strains with a single mismatch without difficulty. At 2 mismatches, each marker 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 main should maintain <3 mismatches on both markers.

According to the most recent in silico analysis performed on 06-Jul-21, with a subsampling of 3878 sequences, mismatches were identified and listed in **Table 11.4**. Despite of the mismatches, a total of 100%, or 3878/3878 sequences, are expected to be detected by the RdRp marker, while 99.97%, or 3877/3878 sequences, are expected to be detected by the gene E. Because the Logix Smart SARS-CoV-2 DS does not differentiate gene RdRp from gene E, the in silico analysis translates to 100% detection rate as a potential severe mismatch on gene E is compensated by the homology of RdRp marker and vice-versa.

Table 11.4 In Silico Inclusivity for Logix Smart SARS-CoV-2 DS

	100 % homology (No mismatches)	**98% homology (1 mismatch)**	**95% homology (2 mismatches)**	**<95% Homology (3+ mismatches)**
RdRp conservation	91.83%	8.17%	0%	0%
E-gene conservation	98.48%	1.52%	0%	0.03%

Evaluation of the impact of the current Variants of Concern (VOC)

Additionally, the global health community, including WHO (World Health Organization), ECDC (European Centre for Disease Prevention and Control), US CDC (Centers for Disease Control and Prevention), among others, have identified certain Variants of Concern (VOC). Co-Diagnostics has the commitment to monitor monthly and report trends of the performance of its COVID-19 test kits as needed.

Since initial design in Feb-2020, none of the mutations related to the lineages B.1.1.7 (alpha), B.1.351 (beta), B.1.427 (epsilon), B.1.429 (epsilon), P.1 (gamma), or C37 (lambda) are predicted to impact the performance of any tests designed and manufactured by Co-Diagnostics. This status has not changed since Feb-2020, even in the most recent in silico analysis as these mutations do not happen in a region targeted by any of the RdRp or E gene CoPrimers.

However, the in silico analysis performed in June/2021 found that one of the mutations leading to the variant Delta (B.1.617.2) is located on the targeted location of one of the RdRp CoPrimers. The mutation is a single nucleotide substitution. As discussed above, 1 mismatch represents a 98% homology of the CoPrimer with the targeted region, and it is not expected to impair the performance of the CoPrimer. Moreover, the Logix Smart SARS-CoV-2 DS combines the RdRp and gene E markers, any impairment of the performance of one marker is compensated by the other, therefore the mutation related to the one single nucleotide in one of the RdRp CoPrimers will not impact the performance of the Logix Smart SARS-CoV-2 DS to detect any of the variants of concern B.1.1.7 (alpha), B.1.351 (beta), B.1.427 (epsilon), B.1.429 (epsilon), P.1 (gamma), B.1.617.2 (delta) or the variant of interest C.37 (lambda) (WHO, 2021) (CDC, 2021) (ECDC, 2021) (GOV.UK, 2021), according to the most current information retrieved from the NextStrain public genomic databases..

In summary, the reasons why the mutations do not impact the performance of Logix Smart SARS-CoV-2 DS are:

a) The Logix Smart SARS-CoV-2 targets 2 different regions of the SARS-CoV-2 virus genome, the gene RdRp and gene E. The amplification signal is generated by both markers. In the event one marker fails due to mutations the other will be responsible by the signal.
b) The single nucleotide mutation on variant of concern Delta is not expected to impair the amplification on the affected RdRp CoPrimer.
c) The performance of the Logix Smart SARS-CoV-2 DS is a composite of the amplification of all its four CoPrimers.

11.4 Analytical Specificity – Cross-reactivity or Exclusivity

11.4.1 Cross-reactivity performed by *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) Database is reviewed consistently to detect potential mutations in the targeted region; 4) The search parameters automatically adjusted for short input sequences and the expect threshold is 1000; 5) 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 11.5**.

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 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 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.

Table 11.5 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	*Corynebacterium diphtheriae*
Human coronavirus NL63	Influenza A & B	*Legionella non-pneumophila*
SARS-coronavirus	Enterovirus	*Bacillus anthracis* (Anthrax)
MERS-coronavirus	Respiratory syncytial virus	*Moraxella catarrhalis*
	Rhinovirus	*Neisseria elongata*
	Chlamydia pneumoniae	*Neisseria meningitides*
	Haemophilus Influenza	Leptospirosis
	Legionella pneumophila	*Chlamydia psittaci*
	Mycobacterium tuberculosis	*Coxiella burnetii* (Q-Fever)
	Streptococcus pneumoniae	*Staphylococcus aureus*
	Streptococcus pyogenes	
	Bordetella pertussis	

	Mycoplasma pneumoniae	
	Pneumocystis jirovecii (PJP)	
	Microbial flora in the human respiratory tract (represented by pooled nasal wash)	
	Candida albicans	
	Pseudomonas aeruginosa	
	Staphylococcus epidermidis	
	Staphylococcus salivarius	

11.4.2 Cross-reactivity performed by wet-test.

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 11.6**. 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 11.6 Logix Smart SARS-CoV-2 DS Exclusivity Testing

Sample	Strain/Isolate	SARS-CoV-2 Cq
SARS-Related Coronavirus 2 (SARS-CoV-2)	USA-WA1/2020	Positive
Human coronavirus OC43	Betacoronavirus 1 OC43	Negative
Human coronavirus HKU1	Human coronavirus HKU1	Negative
Human coronavirus NL63	Human coronavirus NL63	Negative
SARS-coronavirus (2003)	HKU-39849	Positive (Cq 34.37)
MERS-coronavirus	Betacoronavirus England-1	Negative
Human Metapneumovirus (hMPV)	TN/91-316	Negative
Parainfluenza virus 3	C 243	Negative
Enterovirus (e.g., EV68)	F02-3607 Corn	Negative
Respiratory syncytial virus	Long	Negative
Rhinovirus	1059	Negative
Chlamydia pneumoniae	TW-183	Negative
Haemophilus influenzae	Type B	Negative
Legionella pneumophila	Philadelphia 1	Negative
Mycobacterium tuberculosis	H37Rv	Negative
Streptococcus pneumoniae	GA17545	Negative
Streptococcus pyogenes	MGAS1882, serotype M59	Negative
Bordetella pertussis	F	Negative
Mycoplasma pneumoniae	FH of Eaton Agent	Negative
Pneumocystis jirovecii (PJP)	Rat prototype	Negative
Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract*	Not Applicable/Available	Negative
Candida albicans	NIH 3172	Negative
Pseudomonas aeruginosa	R. Hugh 813	Negative
Staphylococcus epidermidis	PCI 1200	Negative
Streptococcus salivarius	DSM 13084	Negative

11.5 Analytical Specificity – Interfering Substances

Common interfering substances potentially found in a clinical sample were tested to establish that the detection of the Logix Smart SARS-CoV-2 DS test kit remained unaffected. Analysis was performed by spiking both positive and negative contrived saliva samples with potentially interfering substances. For endogenous and disinfecting/cleaning substances, the materials were spiked pre-extraction and were spiked at a concentration that is 1-2x the highest concentration that a laboratory would expect to observe among patient specimens submitted for analysis. Exogenous substances were collected in saliva donated by individuals within 15 minutes of having consumed a typical serving of the substance. For the positive samples, the SARS-CoV-2 reference materials were spiked in at 3x LoD or clinical samples with Ct value equivalent to 3x LoD, e.g., Ct ≈ 30. Both positive and negative samples were run in triplicate. Additionally, a positive 'control' sample spiked at 3x LoD without interfering substances was run in triplicate.

Table 11.7 Interfering Substances Testing

Interfering Substance	Concentration	Sample Matrix	Average Cq + S.D.	ΔCq Compared to Control
Human Whole Blood	2.5% v/v	Saliva	37.37 ± 0.55	1.38
Human Genomic DNA	20 ng/µL	Saliva	35.74 ± 0.64	0.75
Human Peripheral Blood Mononuclear Cells (PBMCs)	1.0E+03 cell/µL	Saliva	36.23 ± 1.16	1.24
Coffee	N/A	Saliva	36.45 ± 1.07	1.46
Coke/Diet Coke	N/A	Saliva	35.41 ± 0.63	0.42
Orange Juice	N/A	Saliva	31.12 ± 0.35	-0.87
Mouth Wash	N/A	Saliva	34.53 ± 0.30	-0.46
Toothpaste	N/A	Saliva	33.19 ± 0.18	-1.80
Cough Drops (w/ menthol)	N/A	Saliva	34.99 ± 1.13	0.00
Bleach	2% v/v	Saliva	37.32 ± 1.37	1.33
Disinfecting Wipes	½ in^2	Saliva	38.33 ± 0.42	1.34
Ethanol	7% v/v	Saliva	35.66 ± 0.49	0.67
DNAZap	1% v/v	Saliva	37.41 ± 1.60	1.42
RNAZap	1% v/v	Saliva	34.58 ± 0.39	-0.41

11.6 Clinical Evaluation

Samples were procured and tested by a third-party clinical laboratory. 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 for the study. The remnant positive samples were procured in October/2020, aliquoted, used in a previous study, and adequately maintained at -70ºC controlled storage area. The comparator assay was the laboratory developed test validated internally and routinely used by the clinical laboratory.

The 30 known positive remnant saliva and the fresh 30 negative specimens were treated with the 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-004) 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 validated 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 11.8**.

Table 11.8 Summary of Results for Clinical Evaluation of Logix Smart SARS-CoV-2 DS

		Laboratory's routine LDT (Paired saliva & anterior nasal swab)		Total samples
		Negative	Positive	
Logix Smart SARS-CoV-2 DS (Saliva)	**Negative**	30	0	30
	Positive	1	29	30
Positive Agreement (PPA)		96.7% (29/30)		
Negative Agreement (NPA)		100% (30/30)		

11.7 Performance Summary

Table 11.9 Performance Summary for Logix Smart SARS-CoV-2 DS (COVDS-K-003 / COVDS-K-004)

Application	Qualitative Multiplex real-time RT-PCR test kit for the detection of SARS-CoV-2 RNA targeting two genes, RdRp in the polygene Orf1ab region and gene E, using a sample processing technique that eliminates the use of the nucleic acid extraction/purification process.		
Specifications			
Limit of Detection			
Thermocycler	**Proteinase K solution**	**LoD (>95% detection)**	
CoDx Box Cycler (Co-Diagnostics)	Sample Processing Solution (SPS-DSR01) (Co-Diagnostics, Inc.)	3,000 copies/mL	
MIC qPCR Cycler (BioMolecular Systems)	Sample Processing Solution (SPS-DSR01) (Co-Diagnostics, Inc.)	1,700 copies/mL	
QuantStudio 5 (Thermo Fisher Scientific)	Sample Processing Solution (SPS-DSR01) (Co-Diagnostics, Inc.)	3,500 copies/mL	
CFX 96 Touch (Bio-Rad)	Sample Processing Solution (SPS-DSR01) (Co-Diagnostics, Inc.)	5,000 copies/mL	
ABI 7500 Fast Dx (Thermo Fisher Scientific)	Sample Processing Solution (SPS-DSR01) (Co-Diagnostics, Inc.)	2,000 copies/mL	
	MagMAX Viral/Pathogen Proteinase K (Thermo Fisher Scientific)	1,000 copies/mL	
Analytical Specificity **(Wet test cross reactivity)**	Does not cross-reactive with: Human coronavirus OC43, Human coronavirus HKU1, Human coronavirus NL63, MERS-coronavirus, Human (hMPV), Parainfluenza virus 3, Enterovirus (e.g., EV68), Respiratory syncytial virus, Rhinovirus, *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Bordetella pertussis*, *Mycoplasma pneumoniae*, *Pneumocystis jirovecii* (PJP), microbial flora in the human respiratory tract, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Streptococcus salivarius* It may cross-react poorly with SARS-coronavirus (1) (2003)		

Sensitivity*	96.7%
Specificity*	100.0%
Sample type	Saliva
Time to detection	Approximately 90 minutes, depending on the instrument used
Proteinase K reagent compatibility	•Sample Processing Solution (Co-Diagnostics, Inc.) •MagMAX Viral/Pathogen Proteinase K (Thermo Fisher Scientific)
Thermal cycler compatibility	•CoDx Box Cycler (Co-Diagnostics, Inc.) •MIC qPCR Cycler (BMS, BioMolecular Systems) •QuantStudio 5 (Thermo Fisher Scientific) •CFX96 Touch (Bio-Rad) •ABI 7500 Fast (Thermo Fisher Scientific) The test utilizes the following fluorescent dyes: FAM (green channel): SARS-CoV-2 RNA CF610 (ROX) (red channel): Internal Positive Control (IPC) Human RNaseP.

*Results obtained from clinical evaluation with 30 remnant positive saliva samples, and 30 negative paired saliva and anterior nasal swabs.

12 MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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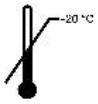
14 LEGEND OF PACKAGE SYMBOLS

IVD	*In vitro* diagnostic medical device		Contains sufficient for 100, 250 or 5,000 reactions
REF	Catalog number		Protect from light
LOT	Batch Code		Temperature limit
CAP	Cap color		Consult Instructions for Use
COMP	Component		Non-Sterile product – Do not sterilize
CONT	Content/Volume		Manufacturer
NUM	Number	EC REP	Authorized Representative in the European Community
	Use-by-date	CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC (IVD)