Oct
2021

Co-Dx

Logix Smart™ ABC (Influenza A/B, SARS-CoV-2)

For *in vitro* diagnostic use

REF **ABC-K-001**

Logix Smart™ ABC (Influenza A/B, SARS-CoV-2)
CO-DIAGNOSTICS, INC.

CE

IVD

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

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1 INTENDED USE

The Logix Smart ABC (Influenza A/B, SARS-CoV-2) is a real-time RT-PCR multi-analyte test using proprietary technology of CoPrimer™ intended for the qualitative simultaneous detection and differentiation of nucleic acid from Influenza A, Influenza B, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), targeting conserved regions of the viruses genomes in lower respiratory tract samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva from individuals suspected of Influenza A, Influenza B, or coronavirus disease COVID-19 and its related conditions.

1.1 Indications for Use

Results are for the identification of Influenza A (gene M, Influenza B, and SARS-CoV-2 RNA. The RNA is generally detectable in lower respiratory tract samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva during the acute phase of infection. Positive results are indicative of the presence of Influenza A, Influenza B, and/or SARS-CoV-2 genomic material; 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.

Negative results do not preclude Influenza A, Influenza B, and/or SARS-CoV-2 infections and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Logix Smart ABC test 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 ABC test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test utilizing the Company's patented CoPrimer technology (Satterfield, 2014) (Poritz & Ririe, 2014). The CoPrimer sets to detect Influenza A (gene (M) Matrix), Influenza B (gene Nonstructural [NS]), and SARS-CoV-2 (gene RdRp and E-gene) in lower respiratory tract samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva from patients who are suspected of Influenza A, Infuenza B, or coronavirus disease 2019 (COVID-19).

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Each **Logix Smart ABC (Influenza A/B, SARS-CoV-2)** consists of the following components:

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

2.1 Principles of Operation

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

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

The sample preparation for PCR requires the samples to be processed to break apart cells and viruses to expose the genetic material. For this process, a commercially available extraction system is used. In this process, the nucleic acids are isolated and purified from the lower respiratory tract fluids (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract fluids (e.g., nasopharyngeal and oropharyngeal swabs), or saliva.

The purified nucleic acid is then plated with the Logix Smart ABC master mix, 10 µl of each. The master mix is pre-mixed and contains the necessary components to perform both the reverse transcription and PCR and does not need to be prepared ahead of time by the user. The plated reactions will then be put in the thermocycler using the following cycling conditions: 15 min at 45°C, 2 min at 95°C, 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 thermocycling for the PCR.

During the PCR, the labeled forward CoPrimer acts as both the forward primer and probe. During the annealing/extension phase of the PCR, the 5’ nuclease activity of Taq polymerase degrades the CoPrimers portion that annealed to the targeted sequence, causing spatial separation between the fluorophore and the quencher, generating a fluorescent signal. With each cycle, additional fluorophore molecules

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are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at the end of each cycle by the real-time thermocycler, specifically the CoDx Box.

See Table 1 for the components included in the test kit.

Table 1

Components Included in the Test Kit

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Brown	Logix Smart ABC Master Mix	MM	ABC-MM-001	Proprietary blend of CoPrimers™ and PCR reagents	1x1000 µL (100 reactions) or 1x25000 µL (2,500 reactions)
Red	Logix Smart ABC Positive Control	PC	ABC-PC-001	Proprietary blend of Influenza A/B, 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)

3 REAGENT STORAGE AND HANDLING

The following list includes information for reagent storage and handling:

- The **Logix Smart ABC (Influenza A/B, SARS-COV-2)** 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 ABC (Influenza A/B, SARS-COV-2)** 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 ABC (Influenza A/B, SARS-COV-2)** 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.

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4 MATERIAL REQUIRED BUT NOT INCLUDED WITH THE TEST

Extraction System required but not included with the test are displayed in Table 2. Thermocyclers validated but not included with the test are displayed in Table 3.

Table 2

Extraction and Automation Systems Validated with the Test

Extraction Reagent		Automation Platform (If applicable)	Manufacturer	Sample Input Volume/Sample Elution Volume
Name	Cat. Number			
QIAamp Viral RNA Mini Kit (Qiagen)	52904 (50 extractions) 52906 (250 extractions)	N/A	Qiagen	200 µL/60 µL

Table 3

Thermocyclers Validated but Not Included with the Test

Thermocycler Machine	Catalog Number	Manufacturer
CoDx Box	MIC-4	Co-Diagnostics, Inc.
Mic qPCR Cycler	MIC-4	BMS, Bio Molecular Systems
PCRmax Eco 48 Real-Time qPCR System	EW-93947-00	PCRmax Limited
CFX 96 Touch Real-Time PCR Detection System	1855195	Bio-Rad

4.1 Consumables Required but Not Provided

Consumables required but not provided include the following:

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

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4.2 Equipment Required but Not Provided

Equipment required but not provided include 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 the extraction
- A PCR workstation, for master mix plating and setup
- An appropriate thermocycler

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 ABC (Influenza A/B, SARS-COV-2) 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 ABC (Influenza A/B, SARS-COV-2)** test kit is provided with the shipment. If not provided with the shipment, the SDS can be retrieved from Co-Diagnostics website at the link: http://co-dx.com/products/diagnostic-solutions/

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- 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://co-dx.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 local regulations, internal validation and procedures for sample selection, collection, transport, and storage, and any other handling procedure.

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

- CDC - https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html
- European CDC - https://www.ecdc.europa.eu/en/novel-coronavirus/laboratory-support
- WHO - https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance

6.1 Lower Respiratory Tract Specimen

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

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

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6.2 Upper Respiratory Tract Specimen

6.2.1 Nasopharyngeal swab and oropharyngeal swab (NP/OP swab): use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing viral transport media. NP and OP specimens should be kept in separate vials. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack.

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

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

6.2.3 Nasopharyngeal wash/aspirate or nasal aspirate: collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on an ice pack. Or collect the sample on a vial with virus transport media which does not demand refrigeration or cold chain transport, it may require validation by the laboratory.

6.3 Saliva

6.3.1 Collect 2-3 mL into a sterile, leak-proof, screw-cap container, or collect saliva up to the fill-line for screw-cap saliva collection kits. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on ice pack. For saliva collection kits, follow the manufacturer's instructions for storage and shipping conditions (CDC, 2020).

6.4 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 influenza A, influenza B, and/or SARS-CoV-2 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).

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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).

6.5 Sample Storage

It is recommended that all specimen types are processed within 48 hours after collection, if storage is needed after 48 hours, it is recommended that samples to be stored frozen, preferably at -70°C (ECDC, 2020). 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).

6.6 Sample Shipping

Specimens known to be, or suspected of, containing influenza A, influenza B, and/or 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.).

7.1 Sample Preparation

The quality of the RNA from the extraction of the sample is essential to the performance of **Logix Smart ABC (Influenza A/B, SARS-COV-2)**. The extraction protocol should be performed following the manufacturer's instructions.

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7.1.1 Extraction of RNA with QIAamp® Viral RNA Mini Kit, cat. no. 52904/52906, Qiagen

The extraction can be performed following the manufacturer's instructions using 140 µL of the sample, and an elution using 60 µL of buffer AVE. For additional sensitivity, load up to 200 µL of patient sample, and increase the volume of Buffer AVL from 560 µL to 800 µL. To ensure the removal of residual wash buffer from the sample prior to elution, an additional centrifugation step (see extraction procedure) using a new collection tube is required.

Wash buffers used in the extraction kit contain ethanol. It is important to eliminate any traces of ethanol before elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

7.2 Logix Smart ABC (Influenza A/B, SARS-COV-2) Reagent Setup

Do the following when performing reagent setup:

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by molecular grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.
- Vortex all **Logix Smart ABC (Influenza A/B, SARS-COV-2)** master mix, PC, nuclease-free water (used as a no template control [NTC]), and sample tubes for 3 seconds and briefly spin 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.

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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 (elution from nucleic acid extraction) or 10 µL of a control (NTC and PC) to the appropriate well(s). At least one positive and one NTC control must be included in each run.

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

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

7.4 qPCR Instrument Setup for the CoDx Box

7.4.1 Contact the Laboratory 801-438-1036 ext. 03 or at www.co-dx.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 settings displayed in Table 4.

Table 4

qPCR Instrument Settings

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

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7.5.2 Program PCR instrument according to the cycling conditions in Table 5.

Table 5

PCR Instrument Cycling Conditions

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

7.5.3 Define the fluorescence detectors (dyes) as displayed in Table 6.

Table 6

Fluorescence Detectors (Dyes) Definitions

Target	Detector Name	Reporter	Quencher
INFA specific RNA	INFA	Quasar® 670	BHQ® - 2
INFB specific DNA	INFB	CAL Flour® Orange 560	BHQ® - 1
SARS-CoV-2 specific RNA	SARS-CoV-2	FAM™	BHQ® - 1
RNaseP specific DNA (IPC)	RNaseP	CAL Flour® Red 610	BHQ® - 2

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

8 DATA ANALYSIS

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.

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Verification and validation studies performed for **Logix Smart ABC (Influenza A/B, SARS-COV-2)** (Catalog number ABC-K-001) were conducted following Good Laboratory Practices for Molecular Biology assays (Viana & Wallis, 2011). If these conditions are not met, the performance will show higher variability due to user errors while experimenting.

8.1 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 RNA), the red channel (monitoring for Influenza A RNA), the yellow channel (monitoring for Influenza B RNA), 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
- Check the box to “Auto Generate Analysis”

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 the SARS-CoV-2 marker in the FAM channel, Influenza A marker in the Quasar 670 (Q670), Influenza B marker in the CF560 channel, and amplification of the internal positive control for RNaseP (IPC) in the CF610 channel. A positive amplification curve looks similar to the purple curve in Figure 1 and should have a Cq value below 40 cycles.

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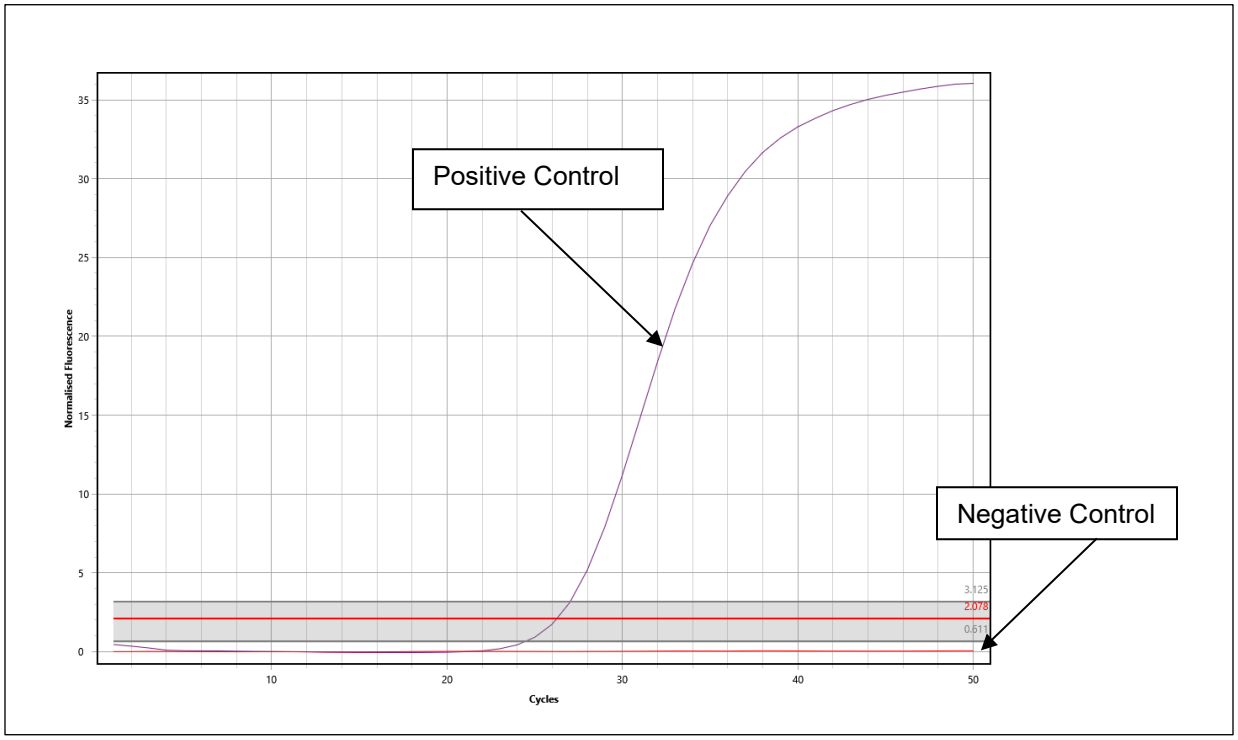
Figure 1

Positive Control (PC) and No Template Control (NTC) Signals for Logix Smart ABC



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 1, as the red line, which is below the threshold area. The threshold area is the grey band with the red line.

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8.4 The Validity of the Diagnostic Test Runs

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

8.4.1 The control conditions displayed in Table 7 must be met.

Table 7

Control Conditions

Control Type	Control Name	Purpose of Control	INF A	INF B	SARS-CoV-2	Internal Control (RNaseP)
ABC Positive Control	INFA (Quasar®670)	Verifies the performance of the master mix	+	+	+	+
	INFB (CF®560)					
	SARS-CoV-2 (FAM™)					
	RNaseP (CF®610)					
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-	-	-

- If controls pass, interpret the sample results.

8.4.2 Invalid Diagnostic Test Run

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

If Internal Positive Control (RNaseP) fails an investigation should be initiated to eliminate possible splashing, pipetting error, or any other laboratory error.

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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 Influenza A (INF A), Influenza B (INF B), or SARS-CoV-2 at or below 40 cycles. Amplification curves greater than 40 cycles for the targets are in the uncertainty zone. The presence of a curve, with a Cq at or below 40 cycles, for a sample for INF A, INF B, and/or COVID-19, indicates a positive result. The amplification of the RNaseP (IPC) shows that the extraction was successful.

A **Negative** result will show no amplification for INF A, INF B, and/or SARS-CoV-2; occasionally amplification greater than 40 cycles may occur in any of the channels. Any amplification curves greater than 40 cycles are in the uncertainty zone and possibly below the limit of detection. Performing an additional run of the same sample or another sample of the patient in the same or following days should be considered. The absence of a curve for INF A, INF B, and/or 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 Table 8.

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Table 8

Interpretation of Results for the Logix Smart ABC

	SARS-CoV-2	Influenza A	Influenza B	Patient Internal Positive Control (RNaseP)	Logix Smart™ ABC Positive Control	No Template Control (NTC): Logix Smart™ ABC Master Mix + Nuclease-Free Water	Result
Instrument Reading		+	+	+	+	-	ABC +
	-	-	-	+	+	-	ABC -
	+	-	-	+	+	-	SARS-CoV-2 +
							INF A -
							INF B -
	-	+	-	+	+	-	SARS-CoV-2 -
							INF A +
							INF B -
	-	-	+	+	+	-	SARS-CoV-2 -
							INF A -
							INF B +
	+	+	-	+	+	-	SARS-CoV-2 +
							INF A +
							INF B -
	-	+	+	+	+	-	SARS-CoV-2 -
							INF A +
							INF B +
	+	-	+	+	+	-	SARS-CoV-2 +
							INF A -
							INF B +
	Any Result (+/-)			-	+	-	INVALID
				+	-	-	See Troubleshooting
				+	+	+	

Anything before 40 cycles is considered a positive reading (+). Anything after 40 cycles is considered a negative reading (-). When possible, always check that the medical history and/or symptoms match with the result prior to treatment.

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9 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Logix Smart ABC (Influenza A/B, SARS-COV-2) test** kit**,** even if the recommended steps for troubleshooting solves the issue. To give feedback please fill out the Customer Feedback Form by visiting https://co-dx.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

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 ABC Positive Control not Amplifying

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

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,

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- **Logix Smart ABC Master Mix** or **Logix Smart ABC 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 re-extracted. If failure of the positive control happens a third time after re-extraction and re-amplification, open a new **Logix Smart ABC Positive Control** or **master mix**, and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 02 or contact us at www.co-dx.com/contact/.

9.3.3 RNaseP (IPC) not Amplifying in Patient Samples

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

- Not enough nuclear material in the patient sample,
- PCR inhibitors such as ethanol and heparin,
- the extraction was performed incorrectly,
- or the extraction kit used is not compatible or has a step that eliminates RNaseP DNA.
- Note: Positive amplification in the any of the target channels indicates a positive result despite the lack of concurrent amplification in the IPC channel. The IPC amplification is dependent on the presence of human genomic DNA (gDNA) in the extraction sample, the amount of which is governed by the type of the patient sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g., CSF, urine, cell lysates, etc.) may not contain the human RNaseP gene.
    - If IPC (CF610 channel) shows a negative result while the target channel(s) shows positive result an internal investigation should be initiated.
    - In the investigation the two possible scenarios should be evaluated:

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- The positive result for INF A, INF B, and/or SARS-CoV-2 channel(s) 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 INF A, INF B, and/or SARS-CoV-2 channel(s) 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.

Fail of any of the controls may indicate that the sample extraction or sample collection have failed. In this scenario a new extraction should be performed, if the IPC persists to be negative with negative INF A, INF B, and 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. 02 or contact us at www.co-dx.com/contact/.

9.3.5 No Template Control Showing Amplification

Amplification of INF A, INF B, and/or 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 re-extracted. If failure of the NTC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, the run should be interpreted as invalid. Please contact Co-Diagnostics Inc. Technical Support by calling 801-438-1036 ext. 02 or at: www.co-dx.com/contact/.

10 LIMITATIONS

Limitations include the following:

- 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 co-dx.com/resources/instructions-for-use/

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- 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 ABC (Influenza A/B, SARS-COV-2)** kit components directly on the specimens collected. Perform an appropriate nucleic acid extraction before using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the SARS-CoV-2, genome covered by this test kit may fail to detect the presence of the pathogens.
- As with any diagnostic test, results of **Logix Smart ABC (Influenza A/B, SARS-COV-2)** kit are to be interpreted with consideration of all clinical and laboratory findings.

11 ANALYTICAL EVALUATION

11.1 Precision

The precision was performed over 5 days with 2 runs, performed in shifts, a day with 2 machines, and two technicians. Samples were prepared by spiking Influenza A, Influenza B, and/or SARS-CoV-2 Amplirun (Vircell) extracted viral genomic RNA controls into confirmed negative clinical matrix (saliva collected with SDNA-1000 Saliva Collection Device (Spectrum Solutions (Utah, USA), Catalog # SDNA-1000). The concentrations for the ‘normal’ and ‘low’ concentrations are based on the LoD and detection rates of ~99% and 95%, respectively. The difference in the total average Cq and average Cq each day should be less than or equal to 2.0 cycles, with variance lower than 5%, and there should be at least a 95% detection rate for all the markers. Results have been found with the acceptance criteria with no shift in cycle higher than 2.0 cycles between days, machines, and operators. The detection was within at least 95%, and the coefficient of variance was less than 5%.

See Table 9 for combined precision results for Logix Smart ABC.

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Table 9

Combined Precision Results for the Logix Smart ABC

	Cq Average	SD	Call Rate	CV%	Marker Detection Rate (%)
Red Channel					
INF A [Normal]	34.21	0.61	20/20	1.79	100%
INF A [Low]	34.99	0.68	20/20	1.95	100%
Combined [Normal]	33.90	0.61	20/20	1.80	100%
Combined [Low]	34.55	0.81	19/20	2.34	95%
Yellow Channel					
INF B [Normal]	29.76	0.50	20/20	1.67	100%
INF B [Low]	30.07	0.53	20/20	1.76	100%
Combined [Normal]	29.58	0.50	20/20	1.71	100%
Combined [Low]	29.92	0.55	19/20	1.84	95%
Green Channel					
COVID [Normal]	33.25	0.59	20/20	1.77	100%
COVID [Low]	33.96	0.62	19/20	1.81	95%
Combined [Normal]	33.48	0.46	20/20	1.37	100%
Combined [Low]	33.65	0.49	20/20	1.45	100%

11.2 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 contrived samples prepared by spiking a reference material in the confirmed negative clinical matrix. For preparing the contrived samples it was used the following reference material:

- Influenza A: A/New Caledonia/20/1999 (H1N1) strain (BEI Resources, catalog # NR-41799)
- Influenza B Virus: B/Malaysia/2506/2004 strain (BEI Resources, catalog # NR-12280)
- SARS-CoV-2: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI Resources, catalog number NR-52287)

The confirmed negative saliva used for the negative matrix was collected with SDNA-1000 Saliva Collection Device (Spectrum Solutions (Utah, USA), Catalog # SDNA-1000). This negative matrix was spiked in after the lysis step of the QIAamp Viral RNA Mini kit (Qiagen, CAT#52906) manufacturer's extraction protocol, using an

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input volume of 200 µL and an elution volume of 60 µL. After the extraction process, the extracts were tested following the Logix Smart ABC protocol. A preliminary LoD experiment was performed using different dilutions with the LoD calculated by probit analysis. Once the LoD range was determined the LoD concentration was confirmed across different thermocyclers with 20 individual samples replicates. The LoD concentration was confirmed with CoDx Box thermocycler (Co-Diagnostics, Inc.) in saliva and sputum (as worst-case for upper and lower respiratory specimen), and confirmed with saliva with CFX96 (Bio-Rad), Eco48 (PCRmax/Cole-Parmer), and Mic qPCR Cycler (Biomolecular Systems). The detection rate was determined at **571.30 $CEID_{50}$/mL or 0.57 $CEID_{50}$/µL** for Influenza A, **60.0 $CEID_{50}$/mL or 0.06 $CEID_{50}$/µL** for Influenza B, and **$1.0x10^3$ copies/mL** for SARS-CoV-2 has been of at least 95% across all machines.

See Table 10 for the verification of the LoD.

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Table 10

Verification of the LoD

Saliva LoD Verification with CoDx Box Thermocycler (Co-Diagnostics)			
	INF A (0.57 $CEID_{50}$/µL)	**INF B (0.06 $CEID_{50}$/µL)**	**SARS-CoV-2 (1.00x10^3 Copies/mL)**
# Detected	19	20	20
# Samples	20	20	20
Detection Rate (%)	95	100	100
Ct Avg.	35.74	35.21	34.81
Sputum LoD Verification with CoDx Box Thermocycler (Co-Diagnostics)			
	INF A (0.57 $CEID_{50}$/µL)	**INF B (0.06 $CEID_{50}$/µL)**	**SARS-CoV-2 (1.00x10^3 Copies/mL)**
# Detected	20	19	20
# Samples	20	20	20
Detection Rate (%)	100	95	100
Ct Avg.	36.00	34.76	35.50
Saliva LoD Verification CFX96 (Bio-Rad)			
	INF A (0.57 $CEID_{50}$/µL)	**INF B (0.06 $CEID_{50}$/µL)**	**SARS-CoV-2 (1.00x10^3 Copies/mL)**
# Detected	20	20	20
# Samples	20	20	20
Detection Rate (%)	100	100	100
Ct Avg.	38.25	37.41	37.70
Saliva LoD Verification with Eco48 (PCRmax/Cole-Parmer)			
	INF A (0.57 $CEID_{50}$/µL)	**INF B (0.06 $CEID_{50}$/µL)**	**SARS-CoV-2 (1.00x10^3 Copies/mL)**
# Detected	19	19	19
# Samples	20	20	20
Detection Rate (%)	95	95	95
Ct Avg.	38.65	38.44	38.19
Saliva LoD Verification Mic qPCR Cycler (Biomolecular Systems)			
	INF A (0.57 $CEID_{50}$/µL)	**INF B (0.06 $CEID_{50}$/µL)**	**SARS-CoV-2 (1.00x10^3 Copies/mL)**
# Detected	19	20	20
# Samples	20	20	20
Detection Rate (%)	95	100	100
Ct Avg.	35.88	34.82	36.93

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11.3 Analytical Specificity – Inclusivity

11.3.1 In Silico Inclusivity for SARS-CoV-2

An alignment was performed with the oligonucleotide CoPrimer sequences of the COVID-19 CoPrimers with all publicly available nucleic acid sequences for SARS-CoV-2 in GenBank, as well as the GISAID database to demonstrate the predicted inclusivity of the Logix Smart ABC (Influenza A/B, 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 4-Feb-2020 with posterior queries performed on March, April, May, and June, July, August, September, and October. Partial and cumulative results are displayed. Sequences were obtained from
https://github.com/nextstrain/ncov/blob/master/data/metadata.tsv

See Table 11 for the in-silico analysis history.

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Table 11

In Silico Analysis History

Date of CoDx's Analysis for RdRp Marker	SARS-CoV-2 samples analyzed (number of sequences in the analyzed subsample)	Sequences in the pool with 100% homology	Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)	Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)	Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)
27-Jan-20	14	14 (100%)	0 (0%)	0 (0%)	0 (0%)
4-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)
17-Mar-20	571	570 (99.8%)	1 (0.2%)	0 (0%)	0 (0%)
6-Apr-20	3639	3634 (99.86%)	5 (0.14%)	0 (0%)	0 (0%)
4-May-20	4468	4459 (99.80%)	9 (0.2%)	0 (0%)	0 (0%)
3-Jun-20	4558	4537 (99.54%)	21 (0.46%)	0 (0%)	0 (0%)
6-Jul-20	11361	11328 (99.71%)	33 (0.29%)	0 (0%)	0 (0%)
10-Aug-20	22054	22012 (99.81%)	42 (0.19%)	0 (0%)	0 (0%)
9-Sep-20	4417	4394 (99.48%)	23 (0.52%)	0 (0%)	0 (0%)
12-Oct-20	5139	5114 (99.51%)	25 (0.49%)	0 (0%)	0 (0%)
Date of CoDx's Analysis for gene E Marker	**SARS-CoV-2 samples analyzed number of sequences in analyzed subsample**	**Sequences in the pool with 100% homology**	**Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)**	**Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)**	**Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)**
4-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)
9-Sep-20	4417	4400 (99.62%)	14 (0.32%)	2 (0.05%)	1 (0.2%)
12-Oct-20	5139	5126 (99.96%)	11 (0.21%)	0 (0%)	2 (0.04%)

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Each marker in Logix Smart ABC is expected to detect strains with a single mismatch without difficulty. At 2 mismatches, each marker in Logix Smart ABC 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.

The alignment data and posterior updated analyses have shown less than three mismatches for both the forward and reverse CoPrimers on 100% of sequences for the RdRp marker and 99.96% of sequences for the E-Gene marker in the NextStrain Global Subsampling of the GISAID database. Therefore, there is a ~0.04% prediction of false-negative results for the E-Gene marker alone and no prediction of false-negative results for both markers together based upon the available data.

11.3.2 In Silico Inclusivity for Influenza A/B

An alignment was performed with the oligonucleotide CoPrimer sequences of the Influenza A and Influenza B CoPrimer sets with all sequences from the Influenza Research Database (restricted to human hosts) as of 02-Jul-2020 (Influenza A) and 21-Jul-2020 (Influenza B) to demonstrate the predicted inclusivity of the Logix Smart ABC (Influenza A/B, SARS-CoV-2). See Table 12 for the in-silico analysis history for Influenza A and Influenza B.

Table 12

In Silico Analysis History for Influenza A and Influenza B

Date of CoDx's Analysis for Influenza A gene M marker	CoPrimer set	Influenza A (number of sequences in analyzed subsample)	Sequences in the pool with 100% homology	Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)	Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)	Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)
02-Jul-20	Forward	**41,352**	40,851 (98.79%)	488 (1.18%)	13 (0.03%)	0 (0%)
02-Jul-20	Reverse	**41,352**	38,763 (93.74%)	2,449 (5.92%)	133 (0.32%)	7 (0.02%)
Date of CoDx's Analysis for Influenza B gene NS marker	**CoPrimer set**	**Influenza B (number of sequences in analyzed subsample)**	**Sequences in the pool with 100% homology**	**Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)**	**Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)**	**Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)**
21-Jul-20	Forward	**12,385**	11,764 (94.99%)	616 (4.97%)	5 (0.04%)	0 (0%)
21-Jul-20	Reverse	**12,385**	12,155	224 (1.81%)	6 (0.05%)	0 (0%)

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			(98.14%)			

The in-silico analysis predicts that the Logix Smart ABC's Influenza A marker is expected to detect >99.66% of the available sequenced Influenza A and Influenza B markers are expected to detect >99.91% of the available sequenced Influenza B. This lower bound was found by adding up the total number of sequences that could have 3+ combined mismatches on the Forward and Reverse by assuming the maximum possible number of mismatches on the opposite CoPrimer. The actual number of combinations will be lower and therefore the percentage of strains expected to be successfully detected is higher than the above-quoted numbers.

11.3.3 Wet-Test Inclusivity for SARS-CoV-2 and Influenza A

Inclusivity wet testing was performed to confirm that the Logix Smart ABC can detect multiple strains/isolates of the targets See Table 13 for the results. Testing was performed by spiking negative saliva extract at 9x, 3x, and 1x LoD, run in quadruplicate. Due to the lack of available reference material the Infuenza B inclusivity wet test has not been performed at this moment.

Table 13

Logix Smart ABC Inclusivity Testing Results

SARS-CoV-2					
Strain/Isolate	**Concentration**	**# Detected**	**# Samples**	**Detection Rate (%)**	**Ct avg.**
Spanish Isolate	9x LoD	4	4	100.00	32.84
	3x LoD	4	4	100.00	34.46
	1x LoD	4	4	100.00	37.31
Germany/BavPat1/2020	9x LoD	4	4	100.00	34.98
	3x LoD	4	4	100.00	36.44
	1x LoD	2	4	50.00	35.67
Italy-INMI1	9x LoD	4	4	100.00	32.17
	3x LoD	4	4	100.00	33.62
	1x LoD	4	4	100.00	35.95
Influenza A					
Strain/Isolate	**Concentration**	**# Detected**	**# Samples**	**Detection Rate (%)**	**Ct avg.**
H1 (A/Brisbane/59/2007)	9x LoD	4	4	100.00	35.54
	3x LoD	4	4	100.00	36.06
	1x LoD	2	4	50.00	37.41
H3 (A/Perth/16/2009)	9x LoD	4	4	100.00	33.49
	3x LoD	4	4	100.00	35.08
	1x LoD	3	4	75.00	36.94

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11.4 Analytical Specificity – Cross-Reactivity – Exclusivity

11.4.1 In Silico Cross Reactivity for SARS-CoV-2

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: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database is reviewed consistently to detect potential mutations in the SARS-CoV-2, Influenza A, and Influenza B targeted regions; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively. 7) BLASTn was run individually for every organism requested listed in Table 14, the list contains microorganisms relevant to the respiratory infections present in oral and samples.

It is expected that the *E* gene marker will efficiently amplify many strains of both Bat SARS-like coronavirus as well as SARS-CoV. 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 (Satterfield, 2014). 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 ABC (Influenza A/B, SARS-COV-2)** 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 ABC (Influenza A/B, SARS-COV-2)** to produce positive results.

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Table 14

Microorganism Included in the Cross-Reactivity In Silico Assessment

High priority pathogens from the same genetic family	High priority organisms likely in the circulating area	Other microorganisms of importance
Human coronavirus 229E	Adenovirus	Influenza C
Human coronavirus OC43	Human Metapneumovirus (hMPV)	Parechovirus
Human coronavirus HKU1	Parainfluenza virus 1-4	*Corynebacterium diphtheriae*
Human coronavirus NL63	Influenza A & B	*Legionella non-pneumophila*
SARS-coronavirus	Enterovirus	*Bacillus anthracis* (Anthrax)
MERS-coronavirus	Respiratory syncytial virus	*Moraxella catarrhalis*
	Rhinovirus	*Neisseria elongata*
	Chlamydia pneumoniae	*Neisseria meningitides*
	Haemophilus Influenza	Leptospirosis
	Legionella pneumophila	*Chlamydia psittaci*
	Mycobacterium tuberculosis	*Coxiella burnetii* (Q-Fever)
	Streptococcus pneumoniae	*Staphylococcus aureus*
	Streptococcus pyogenes	
	Bordetella pertussis	
	Mycoplasma pneumoniae	
	Pneumocystis jirovecii (PJP)	
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract	
	Candida albicans	
	Pseudomonas aeruginosa	
	Staphylococcus epidermidis	
	Staphylococcus salivarius	

11.4.2 In Silico Cross Reactivity for Influenza A/B

11.4.3 Significant homology is defined as 3 or fewer mismatches on a single Forward or Reverse CoPrimer.

- Influenza A Forward CoPrimer:

  No significant homology found with any non-influenza A organisms.

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- Influenza A Reverse CoPrimer:

  No significant homology found with any non-influenza A organisms.

- Influenza B Forward CoPrimer:

  No significant homology found with any non-influenza B organisms.

- Influenza B Reverse CoPrimer:

  No significant homology found with any non-influenza B organisms.

It is not expected that either the Influenza A or the Influenza B markers will cross-amplify with any other human microflora or any other organisms that have been sequenced in the NCBI database.

11.4.4 Wet-Test Exclusivity

Exclusivity wet testing was performed to confirm that the **Logix Smart ABC** does not cross react with non-target organisms. The test was performed by spiking negative sputum or saliva, with non-target organisms, or the non-target organism's extracted genome. The materials that were already extracted were spiked post extraction. Non-target organisms were spiked in at a final concentration of 1e4 copies/rxn (1e6 copies/mL) and run in duplicate. Additionally, to verify that the presence of non-target genomic DNA/RNA does not affect the ability to detect SARS-CoV-2, non-target organisms were spiked in at a final concentration of 1e4 copies/rxn (1e6 copies/mL) and the AMPLIRUN® RNA Controls for the Influenza A, Influenza B, and SARS-CoV-2 was spiked in at 5x LoD and run in triplicate.

The data generated from the specificity-exclusivity runs are summarized below in Table 15. Based on the results, the presence of the non-target organism's genomic material did not significantly affect the amplification of either the *RdRp* target or the *E* gene target ≥ 2 Cq. Additionally, there was no amplification in the reactions that included only the non-target organism, including the SARS-CoV-1 (2003) which has showed some cross-reactivity *in silico* analysis.

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Table 15

Logix Smart ABC (Influenza A/B, SARS-CoV-2) Exclusivity Testing

	Influenza A	Influenza B	SARS-CoV-2	Negative Sample
Human coronavirus OC43	Positive	Positive	Positive	Negative
Human coronavirus HKU1	Positive	Positive	Positive	Negative
Human coronavirus NL63	Positive	Positive	Positive	Negative
SARS-coronavirus	Positive	Positive	Positive	Negative
MERS-coronavirus	Positive	Positive	Positive	Negative
Human Metapneumovirus (hMPV)	Positive	Positive	Positive	Negative
Parainfluenza virus 3	Positive	Positive	Positive	Negative
Influenza A	Positive	Positive	Positive	Negative
Influenza B	Positive	Positive	Positive	Negative
Enterovirus (e.g., EV68)	Positive	Positive	Positive	Negative
Respiratory syncytial virus	Positive	Positive	Positive	Negative
Rhinovirus	Positive	Positive	Positive	Negative
Chlamydia pneumoniae	Positive	Positive	Positive	Negative
Haemophilus influenzae	Positive	Positive	Positive	Negative
Legionella pneumophila	Positive	Positive	Positive	Negative
Mycobacterium tuberculosis	Positive	Positive	Positive	Negative
Streptococcus pneumoniae	Positive	Positive	Positive	Negative
Streptococcus pyogenes	Positive	Positive	Positive	Negative
Bordetella pertussis	Positive	Positive	Positive	Negative
Mycoplasma pneumoniae	Positive	Positive	Positive	Negative
Pneumocystis jirovecii (PJP)	Positive	Positive	Positive	Negative
Pooled human nasal wash	Positive	Positive	Positive	Negative
Candida albicans	Positive	Positive	Positive	Negative
Pseudomonas aeruginosa	Positive	Positive	Positive	Negative
Staphylococcus epidermidis	Positive	Positive	Positive	Negative
Streptococcus salivarius	Positive	Positive	Positive	Negative

11.5 Clinical Evidence

The clinical evidence was established by a mix of clinical samples with complimentary contrived samples on a total of 90 randomized samples. Of those, 30 were remnant SARS-CoV-2 positive clinical samples, 30 negative clinical samples, 15 contrived Influenza A positive samples, and 15 contrived Influenza B positive samples. The contrived samples were prepared using reference material spiked into confirmed negative clinical matrix.

- Influenza A: A/New Caledonia/20/1999 (H1N1) strain (BEI Resources, catalog # NR-41799).

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- Influenza B Virus: B/Malaysia/2506/2004 strain (BEI Resources, catalog # NR-12280).

The contrived samples were made at a variety of concentrations from ~3x LoD to ~4000x LoD. All of the samples were then extracted with the QIAamp Viral RNA Mini Kit (Qiagen, catalog number 52904/52906) and tested with the **Logix Smart ABC** test kit. For comparator assay, one CE Marking registered/EUA-authorized and another EUA-authorized test was comparator for SARS-CoV-2, for the contrived Influenza samples the comparator was an in-house procedure. There was one discrepant result with an overall agreement of 98.89%. The data is summarized in Table 16.

Table 16

Clinical Study with Results for 90 Randomized Samples

Sample	Results Call	Sample Key	Results Match?	Sample	Results Call	Sample Key	Results Match?
1	Negative	Negative	Yes	46	Negative	Negative	Yes
2	Influenza B	Influenza B	Yes	47	Negative	Negative	Yes
3	Influenza B	Influenza B	Yes	48	Influenza B	Influenza B	Yes
4	SARS-CoV-2	SARS-CoV-2	Yes	49	SARS-CoV-2	SARS-CoV-2	Yes
5	Influenza B	Influenza B	Yes	50	SARS-CoV-2	SARS-CoV-2	Yes
6	Negative	Negative	Yes	51	Influenza A	Influenza A	Yes
7	Negative	Negative	Yes	52	SARS-CoV-2	SARS-CoV-2	Yes
8	SARS-CoV-2	SARS-CoV-2	Yes	53	SARS-CoV-2	SARS-CoV-2	Yes
9	Influenza A	Influenza A	Yes	54	Influenza B	Influenza B	Yes
10	Influenza A	Influenza A	Yes	55	Negative	Negative	Yes
11	SARS-CoV-2	SARS-CoV-2	Yes	56	SARS-CoV-2	SARS-CoV-2	Yes
12	Negative	Negative	Yes	57	Influenza B	Influenza B	Yes
13	Negative	Negative	Yes	58	Influenza A	Influenza A	Yes
14	SARS-CoV-2	SARS-CoV-2	Yes	59	SARS-CoV-2	SARS-CoV-2	Yes
15	Influenza B	Influenza B	Yes	60	SARS-CoV-2	SARS-CoV-2	Yes
16	Negative	Negative	Yes	61	Influenza A	Influenza A	Yes
17	Negative	Negative	Yes	62	SARS-CoV-2	SARS-CoV-2	Yes
18	Negative	Negative	Yes	63	Influenza B	Influenza B	Yes
19	Influenza A	Influenza A	Yes	64	Negative	Influenza A	**No**
20	SARS-CoV-2	SARS-CoV-2	Yes	65	Influenza B	Influenza B	Yes
21	SARS-CoV-2	SARS-CoV-2	Yes	66	SARS-CoV-2	SARS-CoV-2	Yes
22	SARS-CoV-2	SARS-CoV-2	Yes	67	Negative	Negative	Yes
23	Negative	Negative	Yes	68	Negative	Negative	Yes
24	Influenza A	Influenza A	Yes	69	Influenza A	Influenza A	Yes
25	Influenza A	Influenza A	Yes	70	Influenza B	Influenza B	Yes

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Sample	Results Call	Sample Key	Results Match?	Sample	Results Call	Sample Key	Results Match?
26	Influenza B	Influenza B	Yes	71	SARS-CoV-2	SARS-CoV-2	Yes
27	Influenza A	Influenza A	Yes	72	Negative	Negative	Yes
28	Influenza B	Influenza B	Yes	73	SARS-CoV-2	SARS-CoV-2	Yes
29	SARS-CoV-2	SARS-CoV-2	Yes	74	Negative	Negative	Yes
30	SARS-CoV-2	SARS-CoV-2	Yes	75	Negative	Negative	Yes
31	Influenza A	Influenza A	Yes	76	Influenza A	Influenza A	Yes
32	SARS-CoV-2	SARS-CoV-2	Yes	77	SARS-CoV-2	SARS-CoV-2	Yes
33	Negative	Negative	Yes	78	Negative	Negative	Yes
34	Negative	Negative	Yes	79	Negative	Negative	Yes
35	Influenza A	Influenza A	Yes	80	SARS-CoV-2	SARS-CoV-2	Yes
36	SARS-CoV-2	SARS-CoV-2	Yes	81	Negative	Negative	Yes
37	SARS-CoV-2	SARS-CoV-2	Yes	82	SARS-CoV-2	SARS-CoV-2	Yes
38	SARS-CoV-2	SARS-CoV-2	Yes	83	Influenza B	Influenza B	Yes
39	Negative	Negative	Yes	84	Negative	Negative	Yes
40	SARS-CoV-2	SARS-CoV-2	Yes	85	Negative	Negative	Yes
41	Negative	Negative	Yes	86	Negative	SARS-CoV-2	**No**
42	Negative	Negative	Yes	87	Negative	Negative	Yes
43	Influenza B	Influenza B	Yes	88	Negative	Negative	Yes
44	Influenza A	Influenza A	Yes	89	Negative	Negative	Yes
45	SARS-CoV-2	SARS-CoV-2	Yes	90	Influenza B	Influenza B	Yes

11.5.1 Diagnostic Accuracy

The number of true positives, false positives, true negatives, and false negatives were collected and sued to calculate the sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), and Matthews correlation coefficient (MCC) were calculated from the totals. See Table 17.

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Table 17

Diagnostic Accuracy of Logix Smart ABC (Influenza A/B, SARS-CoV-2)

Influenza A		Influenza B		SARS-CoV-2	
True Negatives (TN)	30	**True Negatives (TN)**	30	**True Negatives (TN)**	30
False Positives (FP)	0	**False Positives (FP)**	0	**False Positives (FP)**	0
True Positives (TP)	15	**True Positives (TP)**	15	**True Positives (TP)**	30
False Negatives (FN)	1	**False Negatives (FN)**	0	**False Negatives (FN)**	1
Sensitivity	96.774	**Sensitivity**	100	**Sensitivity**	96.774
Specificity	100	**Specificity**	100	**Specificity**	100
Accuracy	0.984	**Accuracy**	1.000	**Accuracy**	0.984
PPV	1.000	**PPV**	1.000	**PPV**	1.000
NPV	0.968	**NPV**	1.000	**NPV**	0.968
MCC	0.953	**MCC**	1.000	**MCC**	0.953

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11.6 Performance Summary

See Table 18 for a performance summary.

Table 18

Performance Summary for Logix Smart ABC (Influenza A/B, SARS-CoV-2)

Logix Smart ABC (Influenza A/B, SARS-COV-2) Performance Characteristics			
Application	**Qualitative Multiplex PCR test, detection for Influenza A, Influenza B, and SARS-CoV-2**		
Limit of Detection	Influenza A	Influenza B	SARS-CoV-2
	571.3 $CEID_{50}$/mL	60.0 $CEID_{50}$/mL	411.2 copies/mL
Sensitivity*	96.78%	100%	96.78%
Specificity*	100%	100%	100%
Sample type	Lower respiratory samples (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory samples (e.g., nasopharyngeal and oropharyngeal swabs), and saliva		
Time to detection	Approximately 90 minutes, depending on the instrument used		
Thermal cycler compatibility	•CoDx Box (Co-Diagnostics, Inc.) •Mic cycler (BMS, Biomolecular Systems) •ECO48 (PCR Max) •CFX96 (Bio-Rad) The test should work with most qPCR systems with the following channel compatibilities: FAM CF560 (VIC) CF610 (ROX) Quasar 670 (Cy5)		
Extraction kit compatibility	QIAamp® Viral RNA Mini Kit (Qiagen, CAT#52904, 52906)		

* Sensitivity based on clinical study of 30 clinical remnant positive samples, 15 contrived Influenza A, and 15 contrived Influenza B, and 30 negative clinical samples.

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12 MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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IVD

CE

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14 LEGEND OF PACKAGE SYMBOLS

See Table 19 for a legend of package symbols.

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Table 19

Legend of Package Symbols

Icon	Description
IVD	*In vitro* diagnostic medical device
REF	Catalog number
LOT	Batch Code
CAP	Cap color
COMP	Component
CONT	Content/Volume
NUM	Number
	Use-by-date
Σ 100	Contains sufficient for 100 tests/ reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
NON STERILE	Non-sterile product - Do not sterilize.
	Manufacturer
EC REP	Authorized representative in the European Community
CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC

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