Simplexa™ COVID-19 & Flu A/B Direct

REF MOL4250
Rev. 01
(English)

A real-time RT-PCR assay intended for the *in vitro* qualitative detection and differentiation of SARS-CoV-2, influenza A & influenza B viral RNA.

For *in vitro* diagnostic use
Rx Only

INTENDED USE

The DiaSorin Molecular Simplexa™ COVID-19 & Flu A/B Direct real-time RT-PCR assay is intended for use on the LIAISON® MDX instrument for the *in vitro* qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus, and influenza B virus in nasopharyngeal swabs (NPS) and nasal swabs (NS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors.

The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, and influenza B infection.

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

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

The Simplexa™ COVID-19 & Flu A/B Direct assay 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.

SUMMARY AND EXPLANATION

SARS-CoV-2 (also called COVID-19 virus) is a beta coronavirus belonging to the family of coronaviruses, named for the crown-like spikes on their surface. There are four main sub-groupings of coronaviruses, known as alpha, beta, gamma, and delta. Common human coronaviruses are 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus) and HKU1 (beta coronavirus), and these usually cause mild to moderate upper-respiratory tract illnesses, like the common cold.[1,2,3] Other human coronaviruses such as MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS) and SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS) have caused more severe respiratory illness with higher rates of morbidity and mortality. The SARS-CoV-2 is a novel coronavirus that causes coronavirus disease 2019, or COVID-19. SARS-CoV-2 caused an outbreak beginning in December 2019 in Wuhan City, Hubei Province, China and has spread globally, being consequently declared a pandemic by the World Health Organization (WHO).[2,4] Patients with COVID-19 have had mild to severe respiratory illness with symptoms of fever, cough and shortness of breath, and many patients have had complications including pneumonia in both lungs.

Influenza is caused by three immunologic types (A, B, and C) of RNA viruses within the *Orthomyxoviridae* family. Influenza A is classified further by describing two viral proteins expressed on its surface, hemagglutinin and neuraminidase. Hemagglutinin facilitates binding of the virus to respiratory epithelial cells, whereas neuraminidase functions to break those bonds with the host cell so that new virions can be released. Seasonal influenza is typically caused by viruses that contain one of three major subtypes of hemagglutinin (H1, H2, or H3) and one of two subtypes of neuraminidase (N1 or N2). Influenza B is not classified into subtypes.[6]

Influenza classically presents with a combination of upper and lower respiratory signs and symptoms, fever, headache, myalgia, and general malaise. Illness can take on a variety of appearances, ranging from isolated respiratory findings that resemble the common cold, to severe pneumonia requiring hospitalization. Persons at higher risk for hospitalization from seasonal influenza include children <2 years of age, adults >65 years of age, and those with significant comorbidities. Influenza may cause exacerbation of underlying medical conditions, such as asthma or congestive heart failure. The duration of illness is typically 2-5 days, but symptoms may last for a week or longer.[7, 8]

PRINCIPLES OF THE PROCEDURE

The Simplexa™ COVID-19 & Flu A/B Direct assay system is a real-time RT-PCR system that enables the direct amplification, detection and differentiation of SARS-CoV-2 RNA, human influenza A (Flu A) virus RNA and human influenza B (Flu B) virus RNA from unprocessed nasopharyngeal swabs (NPS) and nasal swabs (NS) that have not undergone nucleic acid extraction. The system consists of the Simplexa™ COVID-19 & Flu A/B Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ COVID-19 & Flu A/B Direct assay fluorescent probes are used together with corresponding forward and reverse primers to amplify SARS-CoV-2, Flu A, Flu B and internal control RNA targets. For COVID-19 detection, the assay targets two different regions specific to the SARS-CoV-2 genome; the S gene, which encodes the spike glycoprotein, and the ORF1ab region, which encodes well-conserved non-structural proteins and therefore is less susceptible to recombination. For Flu detection, the assay targets conserved regions of influenza A viruses (matrix gene) and influenza B viruses (matrix gene). The assay provides three results; COVID-19 (ORF1ab and/or S gene detection), influenza A viruses (matrix gene detection) and influenza B viruses (matrix gene detection). An RNA internal control is used to detect RT-PCR failure and/or inhibition.

MATERIALS PROVIDED

The DiaSorin Molecular Simplexa™ COVID-19 & Flu A/B Direct kit contains sufficient reagents for 24 reactions. Upon receipt, store at -10 to -30 ºC (do not use a frost-free freezer). Each vial contains sufficient material for one use. Use within 30 minutes of removing from the freezer.

Kit Description

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ COVID-19 & Flu A/B Direct Reaction Mix	MOL4251	REAG	C	RM	Blue	24	1/24	50 µL

Component Description

Kit Component	Contents
Simplexa™ COVID-19 & Flu A/B Direct Reaction Mix (RM)	DNA polymerase, Reverse transcriptase, RNase inhibitor, buffer and dNTPs, encapsulated RNA template, primers and dye-labeled fluorescent probes specific for detection of COVID-19, influenza A, influenza B, and for the RNA Internal Control
Simplexa™ COVID-19 & Flu A/B Direct Barcode Card	Assay specific parameters, lot number, expiration date

Target	Channel	Excitation	Emission	Targeted Gene
Flu A	520	445-505	507-533	matrix
Flu B	560	505-543	547-573	matrix
SARS-CoV-2	610	502-596	597-623	S and ORF1ab
Internal Control "RNA IC"	690	622-658	652-708	N/A

MATERIALS SUPPLIED SEPARATELY

1. Direct Amplification Disc Kit (REF MOL1455)
    a. Direct Amplification Discs for use on the LIAISON® MDX.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. LIAISON® MDX with LIAISON® MDX Studio Software version 1.1 or higher.
2. Simplexa™ COVID-19 & Flu A/B Positive Control Pack (REF MOL4260).
3. 50 µL fixed volume pipette (VWR Signature™ Fixed Volume Ergonomic High-Performance Pipettor Model VWR FE50 or equivalent).
4. Sterile, nuclease-free disposable pipette tips with filters (Extra Long tips ≥ 91 mm are recommended for pipetting directly from primary collection tubes for NPS)
5. Freezer (manual defrost) at -10 to -30 °C (for kit component and specimen frozen storage).
6. Refrigerator at 2 to 8 °C (for specimens).
7. Disposable, powder-free gloves.

RECOMMENDED MATERIALS

1. Universal Transport Media (UTM) to be used as a No Template Control (NTC).

REAGENT HANDLING AND STORAGE

1. Store reagents at -10 to -30 °C (do not use a frost-free freezer).
2. Allow reagents to thaw at room temperature (approximate range 18 to 25 °C) before use.
3. Do not use kits or reagents beyond their expiration dates.
4. After removing Reaction Mix from freezer storage, initiate the test within 30 minutes.
5. Do not vortex the Reaction Mix.
6. Do not refreeze the Reaction Mix.

WARNINGS AND PRECAUTIONS

1. Consult the LIAISON® MDX Hardware Manual for additional warnings, precautions and procedures.
2. The Simplexa™ COVID-19 & Flu A/B Direct real-time RT-PCR assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, influenza A and influenza B, but not for any other viruses or pathogens.
3. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents and equipment. Wash hands thoroughly when finished performing the test.
4. Do not pipette by mouth.
5. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
6. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
7. Treat all specimens and discs as capable of transmitting infectious agents.
8. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.[6, 7]
9. Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results.
10. Assay setup should be performed at room temperature (approximate range 18 to 25 °C).
11. Use fixed volume pipettes or equivalent to transfer sample and Reaction Mix.
12. Avoid touching the underside of the foil that will be in contact with the wells and disc surface.
13. To prevent potentially erroneous results, make sure that the sample and reagent are added to the appropriate input wells.
14. Finish loading and applying adhesive foil cover to one set of Sample and Reaction wells before opening the foil of adjacent set(s) of Sample and Reaction wells.
15. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer.
16. Do not attempt to remove adhesive foil cover from wedges that have been used or attempt to re-use Sample and Reaction ports that have been used in previous runs.
17. Discs may be reused until all 8 wedges have been used. Dispose of used discs without detaching foil cover in biohazardous waste container.
18. After each use store Direct Amplification Disc flat with the numbered foil side up.
19. Store reagents away from light.
20. Reaction Mix contains > 1% glycerol. Upon inhalation or skin contact, first aid measures should be taken.
21. If kit packaging or contents appear to be broken or damaged do not use and contact DiaSorin Molecular. Contact information is located on the last page of this document.
22. The spectral matrix must be installed in each LIAISON® MDX and should not be changed unless an updated Quick Response (QR) code for the instrument is provided by DiaSorin Molecular. The spectral matrix is unique to each LIAISON® MDX. The spectral matrix was provided with the LIAISON® MDX instrument on the cover of the LIAISON® MDX Hardware Manual. If the matrix label will not scan or cannot be found contact DiaSorin Molecular. The contact information is on the last page of this document.
23. Not installing or changing the spectral matrix can result in false results.

24. **INSTRUCTIONS FOR USE**

A. **SPECIMEN COLLECTION AND HANDLING**

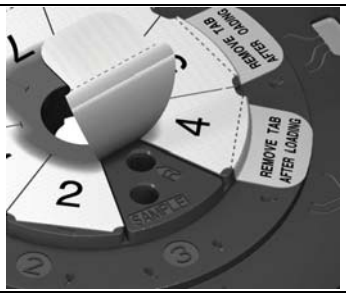
Acceptable specimen types include nasopharyngeal swabs (NPS) and nasal swabs (NS) in 3mL Universal Transport Media (UTM) or BD Universal Viral Transport (UVT) or equivalent, Copan ESwab™ (Liquid Amies), or saline (0.9% sodium chloride in water). Use only authorized swabs with a synthetic tip (e.g. Dacron, nylon, or rayon) and an aluminum or plastic shaft. Do not use calcium alginate swabs, as they may contain substances that inhibit PCR testing.

Specimens should be transported on ice and stored at 2 to 8 °C for up to 3 days post collection. If there is a greater than 3 days delay before processing of the specimen, store specimen at ≤ -70 °C in accordance with the transport media instructions for use.

B. **REAL-TIME PCR INSTRUMENT SETUP**
Refer to the LIAISON® MDX Operator Manual for details on how to configure the LIAISON® MDX Studio Software to add an assay definition, set up and analyze runs on the LIAISON® MDX instrument.

C. **DIRECT AMPLIFICATION DISC LOADING AND REAL-TIME PCR AMPLIFICATION**
NOTE: No sample extraction is needed prior to PCR amplification step.

1. Select samples that need to be tested.
2. Thaw Reaction Mix vials at room temperature (approximate range 18 to 25 °C). Thaw one Reaction Mix vial for each sample or control to be tested.
3. Scan the barcode on the Simplexa™ COVID-19 & Flu A/B Direct Reaction Mix vial or barcode card.
4. Scan the disc barcode on the Direct Amplification Disc (DAD).
5. Scan or type in each sample identifier.
6. For one wedge at a time, peel the adhesive foil back to expose the Reaction (R) and Sample (SAMPLE) wells without completely removing the adhesive foil cover (Figure 1 & 2). Avoid touching the underside of the foil that will be in contact with the wells and disc surface.
7. Ensure that the Reaction Mix is completely thawed. Briefly spin down the tubes as needed. (Do not vortex the Reaction Mix).
8. Use the fixed volume pipette to transfer 50 µL of the Reaction Mix into Reaction (R) well.
9. Briefly pulse vortex the sample. Use the fixed volume pipette to transfer 50 µL of sample or control; pipette sample or control into Sample well (SAMPLE).
10. Cover the wedge sealing the wells with the peeled adhesive foil, pressing down firmly near the edge of the wedge.
11. Tear off the tab portion of the foil cover along the perforation.
12. Repeat steps 6 to 11 for the next sample(s).
13. Load the sealed Direct Amplification Disc into the LIAISON® MDX and start the run.

Figure 1 - Disc with pre-use foil lifted from Reaction [R] and Sample [SAMPLE] Wells for wedge #3	**Figure 2 – Reaction [R] and Sample [SAMPLE] Wells**
	

NOTES *(for informational purposes - no user action/interpretation required)*:
1. DiaSorin Molecular kits may contain version numbers for Assay Definitions. If the version number exists, it will be appended to the Assay Definition i.e. 'Sample IVD Assay.2'. When multiple versions exist, the software automatically uses the assay definition associated with the scanned lot number.

QUALITY CONTROL
Simplexa™ COVID-19 & Flu A/B Positive Control Pack (MOL4260) may be used as an external control for QC testing, training or proficiency testing. Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice. Refer to the Simplexa™ COVID-19 & Flu A/B Positive Control Pack (IFUC.CE.MOL4260) for instructions on running the positive control.

Expected Control Results

Control Type	COVID-19	Flu A	Flu B	RNA Internal Control (RNA IC)
Simplexa™ COVID-19 & Flu A/B Direct Positive Control[a]	Detected	Detected	Detected	Not applicable[b]
No Template Control (NTC)	Not Detected	Not Detected	Not Detected	Valid

[a] Typical Ct values for the Positive Control range between 24-30.
[b] Detection of the Simplexa™ RNA Internal Control (RNA IC) is not required for a valid result.

RESULTS
Upon completion of the run, the software automatically interprets and displays results.

1. For each accession ID (Sample ID) entered, the software displays a result ("Detected", "Not Detected" or "Invalid") for COVID-19, Flu A and Flu B.

Results	Interpretation
Detected	Result indicates the presence of COVID-19 and/or Flu A and/or Flu B in the patient sample.
Not Detected	Result indicates the absence of COVID-19 and/or Flu A and/or Flu B in the patient sample.
Invalid	Result indicates inability to conclusively determine presence or absence of COVID-19, Flu A and Flu B in the patient sample. This result may be due to 1) Internal Control (IC) failure, or 2) failure to detect sufficient specimen volume. The sample needs to be retested. See "Invalid Results" section below.
EC500	Data processing error due to noise, weak or late amplification in the signal. Repeat the sample. If the problem persists, contact Technical Service.
EC505	Insufficient information to determine whether amplification was present. If the problem persists, contact Technical Service.
EC515	Internal control amplification is not within specification. Result is invalid, repeat the sample. If the problem persists, contact Technical Service.

1. Print the report as needed.
2. Export the results as needed.

INVALID RESULTS
In case of an "Invalid" result or an error code, retest the sample with a new reaction mix vial from the same kit or a new kit. If the problem is unresolved, contact DiaSorin Molecular Technical Services Department.

LIMITATIONS
1. For use with Interim Order Authorization Approval 333538 only.
2. For *in vitro* diagnostic use only.
3. For professional and prescription use only.
4. Results from this test must be considered in conjunction with the clinical history, epidemiological data, and other laboratory information available to the clinician evaluating the patient.
5. The detection of viral nucleic acid is dependent upon proper sample collection, transport, handling, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
6. The prevalence of viral infections may affect the test's predictive value.
7. Negative results do not rule out COVID-19, Flu A, or Flu B infections and should not be used as the sole basis for treatment or other patient management decisions.
8. False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
9. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
10. A positive result by this test cannot rule out infections caused by other viral or bacterial pathogens. Viral nucleic acids may persist *in vivo* independent of virus viability. Detection of target analyte(s) does not imply that the corresponding viruses are infectious or are the causative agent for clinical symptoms.
11. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
12. The performance of this test has not been established for screening of blood or blood products for the presence of COVID-19, influenza A or influenza B.
13. The performance of this test has been evaluated for use with human specimen material only.
14. The performance of this test has not been established for immunocompromised individuals.
15. The performance of this test has not been established for patients without symptoms of viral respiratory tract infection.
16. The performance of this test has not been established for monitoring treatment of COVID-19, influenza A or influenza B, or infection.
17. The performance of this test has not been established for individuals who have received an inhaled influenza vaccine.
18. The performance of this test has not been established for use in donor screening tests.
19. The performance of this test has not been evaluated for sample types other than nasopharyngeal and nasal swabs.
20. The performance of this test has not been established for individuals using throat lozenges or products containing menthol.
21. The performance of this device has not been assessed in a population vaccinated against COVID-19
22. The performance of the device has not been assessed on specimens from individuals who have been known to be infected with emerging variants of SARS-CoV-2 of public health concern.

23. Use of 1 mL transport media may result in an increased rate of Internal Control (IC) failures with some patient specimens. This may be due to a higher concentration of inhibitors present when a patient's nasopharyngeal or nasal swab is placed into 1 mL transport media compared to 3 mL transport media. Any 1 mL patient specimens collected may be diluted to a 3 mL final volume (using equivalent transport media) to reduce the amount of inhibitors present.
24. Information on the kit barcode can only be transferred into the LIAISON® MDX Studio Software through a bar-code scanner. If the scanner is not working, or if you are unable to transfer the information for any reason, contact DiaSorin Molecular Technical Services Department.

PERFORMANCE CHARACTERISTICS

CLINICAL EVALUATION

The performance of Simplexa™ COVID-19 & Flu A/B Direct was evaluated using retrospective, pre-selected positive and negative nasopharyngeal swab (NPS) and nasal swab (NS) specimens from human patients with signs and symptoms of respiratory tract infection. The samples were collected from seven (7) external sites, six (6) were US sites and one (1) outside US (OUS) site. The US collection sites were from six (6) different geographical locations. The samples were collected from March 24, 2015 to February 24, 2021. The Simplexa™ COVID-19 & Flu A/B Direct testing and influenza comparator testing was performed from May 14, 2021 to May 18, 2021 at the internal site at DiaSorin Molecular, LLC, Cypress, CA. SARS-CoV-2 comparator testing was performed at an external site from October 26, 2020 to April 24, 2021.

Tables 1-3 below show a comparison of the Simplexa™ COVID-19 & Flu A/B Direct assay and comparator assay results. The positive percent agreement (PPA) and negative percent agreement (NPA) is based on a total of one hundred eighty-six (186) enrolled specimens.

Table 1. Simplexa™ COVID-19 & Flu A/B Direct vs. Real Time RT-PCR Diagnostic Assay – SARS-CoV-2 target

Simplexa™ COVID-19 & Flu A/B Direct (MOL4250)	FDA Emergency Use Authorized SARS-CoV-2 Real-Time RT-PCR Comparator		Total
	Detected	Not Detected	
Detected	55	1	56
Not Detected	2	128	130
Total	57	129	186
	PPA: 96.5% (55/57) 95% CI: 88.1% to 99.0%	**NPA:** 99.2% (128/129) 95% CI: 95.7% to 99.9%	

Table 2. Simplexa™ COVID-19 & Flu A/B Direct vs. Simplexa™ Flu A/B & RSV Direct Gen II – influenza A target

Simplexa™ COVID-19 & Flu A/B Direct (MOL4250)	FDA Cleared NAAT Comparator		Total
	Detected	Not Detected	
Detected	49	2	51
Not Detected	2	133	135
Total	51	135	186
	PPA: 96.1% (49/51) 95% CI: 86.8% to 98.9%	**NPA:** 98.5% (133/135) 95% CI: 94.8% to 99.6%	

Table 3. Simplexa™ COVID-19 & Flu A/B Direct vs. Simplexa™ Flu A/B & RSV Direct Gen II – influenza B target

Simplexa™ COVID-19 & Flu A/B Direct (MOL4250)	FDA Cleared NAAT Comparator		Total
	Detected	Not Detected	
Detected	30	0	30
Not Detected	0	156	156
Total	30	156	186
	PPA: 100.0% (30/30) 95% CI: 88.6% to 100.0%	**NPA:** 100.0% (156/156) 95% CI: 97.6% to 100.0%	

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) of the Simplexa™ COVID-19 & Flu A/B Direct assay in nasopharyngeal swabs (NPS) was determined to be the lowest detectable concentration of quantitated inactivated tittered viral stocks (copies/mL) at which ≥ 95% of all replicates tested positive. Two (2) strains of influenza A, two (2) strains of influenza B and one strain of SARS-CoV-2 were tested. Initially, the tentative LoD was identified with serial dilutions of the viral stocks in negative NPS matrix tested in five (5) replicates during design and development. The lowest concentration at which all replicates were positive was interpreted as the tentative LoD. The LoD was then confirmed by testing twenty (20) replicates with concentrations at the tentative limit of detection. The final LoD was confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity.

LoD results are shown in Table 4 below.

Table 4. Simplexa™ COVID-19 & Flu A/B Direct Limit of Detection

Virus Strain	LOD (copies/mL)
Influenza A/Hong Kong/8/68	1000
Influenza A/Michigan/45/2015	500
Influenza B/Phuket/3073/2013	750
Influenza B/Malaysia/2506/2004	500
SARS-CoV-2 (USA-WA1/2020)	500

ANALYTICAL REACTIVITY/CROSS REACTIVITY

Analytical Reactivity/Inclusivity – COVID-19
An *in-silico* inclusivity analysis of the Simplexa™ COVID-19 primers and probes in the Simplexa™ COVID-19 & Flu A/B Direct assay was performed. All primer and probe sets designed for detection of the ORF1ab and S gene were tested against the complete available SARS-CoV-2 genome sequences. The analysis demonstrated that the regions recognized by the designed primers and probes have very high homology (>98%) with all available SARS-CoV-2 sequences from the Global Initiative on Sharing Avian Influenza Data (GISAID) databases. The low frequency (≤0.1%) of the variants in the primer regions and probe region found during the clinical strain monitoring suggests that they are likely due to sequencing errors or random unique mutations present in few clinical samples and therefore they are not widely present in the analyzed population. Considering that the Simplexa™ COVID-19 & Flu A/B Direct assay is designed for redundancy with detection of SARS-CoV-2 by both ORF1ab and S gene targets, the risk for not detecting SARS-CoV-2 when present in a patient sample is low.. The results are shown in Table 5.

Table 5. Simplexa™ COVID-19 & Flu A/B Direct *In-silico* inclusivity

Database	Identity to ORF1ab design	Identity to S gene design
GISAID	703754 / 709343 (99.2%)	720766 / 730972 (98.6%)

Analytical Reactivity - Influenza
Analytical reactivity was evaluated with nasopharyngeal swab specimens in UTM for the Simplexa™ COVID-19 & Flu A/B Direct assay. A total of 63 influenza A strains and 21 influenza B strains were evaluated. Quantified viral material was spiked into negative NPS matrix at the concentrations below and assayed in triplicate. The results are shown in Tables 6 and 7.

Additionally, all of the influenza strains in the CDC panels for the past three (3) years were reviewed and tested (concentrations as shown in Table 6 & 7). The CDC panels were 100% detected with the Simplexa™ COVID-19 & Flu A/B Direct assay and detailed in Tables 8 - 10.

Table 6. Simplexa™ COVID-19 & FLU A/B Direct Analytical Reactivity Results - Flu A

Organism	Tested Concentration*	% Detected
A/Anhui/1/2013	1:100,000 Dilution	100.0% (3/3)
A/black-legged kittiwake/Quebec/02838-1/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Brisbane/02/2018	100 EID_{50}/mL	100.0% (3/3)

A/Brisbane/10/07	100 U/mL	100.0% (3/3)
A/Brisbane/59/07	100 U/mL	100.0% (3/3)
A/American green-winged teal/Mississippi/300/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/California/02/2014	100 $TCID_{50}$/mL	100.0% (3/3)
A/California/4/2009	100 $TCID_{50}$/mL	100.0% (3/3)
A/California/7/2009	100 $TCID_{50}$/mL	100.0% (3/3)
A/chicken/Germany/N/49	100 $CEID_{50}$/mL	100.0% (3/3)
A/chicken/Vietnam/NCVD-016/2008(H5N1)-PR8-IDCDC-RG12	1:100,000 Dilution	100.0% (3/3)
A/Christ Church/16/2010	1,000 EID_{50}/mL	100.0% (3/3)
A/duck/Chabarovsk/1610/1972	100 $CEID_{50}$/mL	100.0% (3/3)
A/duck/Czechoslovakia/1956	5,000 $CEID_{50}$/mL	100.0% (3/3)
A/duck/Wisconsin/480/1979	100 $CEID_{50}$/mL	100.0% (3/3)
A/Egypt/N03072/2010(H5N1)-PR8-IDCDC-RG29	1:100,000 Dilution	100.0% (3/3)
A/Guangdong-Maonan/1536/2019	100 EID_{50}/mL	100.0% (3/3)
A/Hawaii/15/2001	100 $CEID_{50}$/mL	100.0% (3/3)
A/Hong Kong/2671/2019	100 EID_{50}/mL	100.0% (3/3)
A/Hong Kong/4801/2014	100 TCID50/mL	100.0% (3/3)
A/Hong Kong/33982/2009(H9N2)-PR8-IDCDC_RG26	100 $CEID_{50}$/mL	100.0% (3/3)
A/Hubei/1/2010(H5N1)-PR8-IDCDC-RG30	1:100,000 Dilution	100.0% (3/3)
A/India/NIV/2006(H5N1)-PR8-IBCDC-RG7	1:100,000 Dilution	100.0% (3/3)
A/Indiana/08/2011	100 TCID50/mL	100.0% (3/3)
A/Kansas/14/2017	100 EID_{50}/mL	100.0% (3/3)
A/mallard/Netherlands/12/2000(H7N7)/PR8-IBCDC-1	1:100,000 Dilution	100.0% (3/3)
A/mallard/Illinois/10OS4334/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/mallard/Wisconsin/4218/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/mallard/Wisconsin/4230/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Massachusetts/15/2013	100 $CEID_{50}$/mL	100.0% (3/3)
A/Mexico/4108/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Minnesota/11/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/Minnesota/19/2011	100 $CEID_{50}$/mL	100.0% (3/3)
A/New Caledonia/20/99	100 $TCID_{50}$/mL	100.0% (3/3)
A/New York/18/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/New York/55/2004	100 $CEID_{50}$/mL	100.0% (3/3)
A/NY/02/09	100 $TCID_{50}$/mL	100.0% (3/3)
A/Ohio/02/2012	100 $CEID_{50}$/mL	100.0% (3/3)
A/Perth/16/2009	100 EID_{50}/mL	100.0% (3/3)

A/pheasant/New Jersey/1355/1998(H5N2)-PR8-IBCDC-4	1:100,000 Dilution	100.0% (3/3)
A/Port Chalmers/1/1973	100 $TCID_{50}$/mL	100.0% (3/3)
A/PR/8/34	100 $TCID_{50}$/mL	100.0% (3/3)
A/quail/Italy/1117/1965	100 $CEID_{50}$/mL	100.0% (3/3)
A/red knot/Delaware Bay/240/1994	100 $CEID_{50}$/mL	100.0% (3/3)
A/red knot/Delaware/541/1988	1,000 $CEID_{50}$/mL	100.0% (3/3)
A/redhead/Alberta/192/2002	100 $CEID_{50}$/mL	100.0% (3/3)
A/Rhode Island/01/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/Santiago/7981/2006	100 $CEID_{50}$/mL	100.0% (3/3)
A/shorebird/Delaware Bay/211/1994	1,000 $CEID_{50}$/mL	100.0% (3/3)
A/shorebird/Delaware/172/2006	1,000 $CEID_{50}$/mL	100.0% (3/3)
A/Singapore/INFIMH-16-0019/2016	100 $TCID_{50}$/mL	100.0% (3/3)
A/Solomon Island/3/2006	100 $TCID_{50}$/mL	100.0% (3/3)
A/Swine/1976/31	100 U/mL	100.0% (3/3)
A/Swine/Iowa/15/30	100 U/mL	100.0% (3/3)
A/swine/Ohio/09SW1477/2009	100 $TCID_{50}$/mL	100.0% (3/3)
A/swine/Ohio/09SW83E/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Switzerland/9715293/2013	100 $CEID_{50}$/mL	100.0% (3/3)
A/Taiwan/42/06	100 U/mL	100.0% (3/3)
A/turkey/Massachusetts/3740/1965	2,000 $CEID_{50}$/mL	100.0% (3/3)
A/turkey/Virginia/4529/2002 (H7N2)xPR8-IBCDC-5	1:100,000 Dilution	100.0% (3/3)
A/Texas/50/2012	100 $TCID_{50}$/mL	100.0% (3/3)
A/Wisconsin/67/05	100 $TCID_{50}$/mL	100.0% (3/3)
A/WS/33	100 $TCID_{50}$/mL	100.0% (3/3)

*$TCID_{50}$/mL = Tissue Culture Infectious Dose
$CEID_{50}$/mL = Chicken Embryo Infectious Dose
EID_{50}/mL = Egg Infectious Dose

Table 7. Simplexa™ COVID-19 & Flu A/B Direct Analytical Reactivity Results - Flu B

Organism	Tested Concentration*	% Detection
B/Brisbane/33/2008	100 $CEID_{50}$/mL	100% (3/3)
B/Brisbane/60/2008	100 U/mL	100% (3/3)
B/Christchurch/33/2004	100 $TCID_{50}$/mL	100% (3/3)
B/Colorado/06/2017	100 $TCID_{50}$/mL	100% (3/3)
B/Florida/02/2006	100 U/mL	100% (3/3)
B/Florida/04/2006	100 U/mL	100% (3/3)
B/Florida/07/04	100 U/mL	100% (3/3)

B/Great Lakes/1739/54	100 U/mL	100% (3/3)
B/Guangdong-Liwan/1133/2014	1,000 $CEID_{50}$/mL	100% (3/3)
B/Maryland/1/59	100 $TCID_{50}$/mL	100% (3/3)
B/Massachusetts/02/2012	100 $TCID_{50}$/mL	100% (3/3)
B/Michigan/09/2011	100 EID_{50}/mL	100% (3/3)
B/Nevada/03/2011	100 $CEID_{50}$/mL	100% (3/3)
B/New Hampshire/01/2016	100 EID_{50}/mL	100% (3/3)
B/Panama/45/90	100 U/mL	100% (3/3)
B/Texas/02/2013	100 $TCID_{50}$/mL	100% (3/3)
B/Texas/81/2016	100 EID_{50}/mL	100% (3/3)
B/Utah/09/2014	100 $CEID_{50}$/mL	100% (3/3)
B/Victoria/304/2006	100 $CEID_{50}$/mL	100% (3/3)
B/Washington/02/2019	100 EID_{50}/mL	100% (3/3)
B/Wisconsin/01/2010	100 $CEID_{50}$/mL	100% (3/3)

*$TCID_{50}$/mL = Tissue Culture Infectious Dose
$CEID_{50}$/mL = Chicken Embryo Infectious Dose
EID_{50}/mL = Egg Infectious Dose

Table 8. 2018-2019 CDC panel Flu A and Flu B Strains Tested with Simplexa™ COVID-19 & Flu A/B Direct

Virus	Subtype	Organism
Flu A	A (H3N2)	A/Perth/16/2009
		A/Singapore/INFIMH-16-0019/2016*
	A (H1N1) pdm09	A/California/07/2009
		A/Michigan/45/2015*
Flu B	B (Victoria lineage)	B/Brisbane/60/2008
		B/Colorado/06/2017*
	B (Yamagata lineage)	B/Wisconsin/01/2010
		B/Phuket/3073/2013*

*WHO recommended vaccine strains

Table 9. 2019-2020 CDC panel Flu A and Flu B Strains Tested with Simplexa™ COVID-19 & Flu A/B Direct

Virus	Subtype	Organism
Flu A	A (H3N2)	A/Perth/16/2009
		A/Kansas/14/2017*
	A (H1N1) pdm09	A/Christ Church/16/2010
		A/Brisbane/02/2018*
Flu B	B (Victoria lineage)	B/Michigan/09/2011
		B/Colorado/06/2017*
	B (Yamagata lineage)	B/New Hampshire/01/2016
		B/Phuket/3073/2013*

*WHO recommended vaccine strains

Table 10. 2020-2021 CDC panel Flu A and Flu B Strains Tested with Simplexa™ COVID-19 & Flu A/B Direct

Virus	Subtype	Organism
Flu A	A (H3N2)	A/Perth/16/2009
		A/Hong Kong/2671/2019*
	A (H1N1) pdm09	A/Christ Church/16/2010
		A/Guangdong-Maonan/1536/2019*
Flu B	B (Victoria lineage)	B/Michigan/09/2011
		B/Washington/02/2019*
	B (Yamagata lineage)	B/Texas/81/2016
		B/Phuket/3073/2013*

*WHO recommended vaccine strain

Cross-Reactivity (Analytical Specificity)

Cross-reactivity of the Simplexa™ COVID-19 & Flu A/B Direct assay was evaluated by testing whole organisms or purified nucleic acid from other organisms. Specimens for laboratory testing were prepared by spiking cultured isolates/inactivated organisms/purified nucleic acids (whole genome) into negative matrix (NPS) and determining cross reactivity based on three replicates. RNasin® was added to NPS for specimens containing extracted RNA. Results from cross-reactivity testing are summarized in Table 11. Four (4) additional organisms were not available for testing. BLAST *in silico* analysis showed that detection of *Bacillus anthracis*, *Chlamydophila psittaci*, Influenza C, and *Pneumocystis jirovecii* is not expected due to total percent homology of ≤ 78% for each of the target primers and probe.

Table 11. Simplexa™ COVID-19 & Flu A/B Direct Cross-Reactivity Results

Organism	Tested Concentration[1]	COVID-19	Flu A	Flu B
Adenovirus Type 1	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Adenovirus Type 7A	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Bordetella pertussis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Candida albicans	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Chlamydia pneumoniae	1 x 10^6 IFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Coxiella burnetii	1 x 10^6 genome copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Cytomegalovirus	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Enterovirus Type 68	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Enterovirus Type 71	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Epstein-Barr Virus	1 x 10^5 copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Escherichia coli O157:H7	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Haemophilus influenzae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

Human Coronavirus 229E*	1 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus NL63*	1 x 10^4 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Metapneumovirus 9*	1 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Lactobacillus plantarum,17-5	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Legionella longbeachae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Legionella pneumophila	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Leptospira interrogans	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Measles	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
MERS-Coronavirus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Moraxella catarrhalis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Mumps	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Mycobacterium tuberculosis Genomic DNA	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Mycoplasma pneumoniae	1 x 10^6 CCU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Neisseria elongata	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Neisseria meningitidis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 1	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 2	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 3	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 4	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parechovirus Type 3	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Rhinovirus 1A*	1 x 10^4 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
RSV-A	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
RSV-B	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Staphylococcus aureus	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

Staphylococcus epidermidis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Streptococcus pyogenes	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus RNA HKU1	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Genomic DNA (Leukocytes)	1 x 10^6 cells/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Pooled Human Nasal Wash	1:1 dilution	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
SARS-COV1 Synthetic RNA	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

*A lower concentration was tested due to inability to obtain stock material with high titer, BLAST analysis showed total homology to target primers and probes was ≤ 67%

[1]CCU/mL = Color Changing Units/milliliter

CFU/mL = Colony Forming Units/milliliter

IFU/mL = Infectious Units/milliliter

U/mL = Units/milliliter

$TCID_{50}$/mL = Tissue Culture Infectious Dose

INHIBITION BY OTHER MICROORGANISMS

The Simplexa™ COVID-19 & Flu A/B Direct assay was evaluated by testing the ability to identify SARS-CoV-2, influenza A virus, and influenza B virus, when other potentially inhibitory organisms were present. Specimens were prepared by spiking cultured isolates/inactivated organisms/purified nucleic acids (whole genome) at a minimum of 10^6 CFU/ml (or higher) for bacteria, and 10^5 $TCID_{50}$/mL or PFU/mL (or higher) for viruses into negative matrix (NPS) in the presence of a low dose (1-2X LOD) of the three targets (COVID-19 (2X LOD; 1000 copies/mL), Flu A (1X LOD; 1000 copies/mL) and Flu B (1X LOD; 500 copies/mL) of viral particles and determining microbial inhibition based on three replicates. For organisms not titered in CFU/mL or $TCID_{50}$/mL, other industry acceptable units were used as indicated. RNasin® was added to UTM for specimens containing extracted RNA. The panel of forty-seven (47) potentially inhibitory organisms was individually spiked into a pool with a low concentration influenza A (Influenza A/Hong Kong/8/68), influenza B (Influenza B/Malaysia/2506/2004) and COVID-19 (2019-nCoV/USA-WA1/2020). Samples were assayed in triplicate to screen for inhibition. No inhibition by other organisms was observed for COVID-19, influenza A or influenza B at the concentrations indicated in Table 12.

Table 12. Simplexa™ COVID-19 & Flu A/B Direct – Microbial Inhibition Results

Organism	Tested Concentration[1]	% Detection		
		COVID-19	Flu A	Flu B
Adenovirus Type 1	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Adenovirus Type 7A	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Bordetella pertussis	1 x 10^6 CFU/mL	100%	100%	100%
Candida albicans	1 x 10^6 CFU/mL	100%	100%	100%
Chlamydia pneumoniae	1 x 10^6 IFU/mL	100%	100%	100%
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	100%	100%	100%
Coxiella burnetii	1 x 10^6 genome copies/mL	100%	100%	100%
Cytomegalovirus	1 x 10^5 U/mL	100%	100%	100%

Enterovirus Type 68	1 x 10^5 U/mL	100%	100%	100%
Enterovirus Type 71	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Epstein-Barr Virus	1 x 10^5 copies/mL	100%	100%	100%
Escherichia coli O157:H7	1 x 10^6 CFU/mL	100%	95%	100%
Haemophilus influenzae	1 x 10^6 CFU/mL	100%	100%	100%
Human Coronavirus 229E*	1 x 10^4 $TCID_{50}$/mL	100%	100%	100%
Human Coronavirus NL63*	1 x 10^4 U/mL	100%	100%	100%
Human Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Human Metapneumovirus 9*	1 x 10^4 $TCID_{50}$/mL	100%	100%	100%
Lactobacillus plantarum,17-5	1 x 10^6 CFU/mL	100%	100%	100%
Legionella longbeachae	1 x 10^6 CFU/mL	100%	100%	100%
Legionella pneumophila	1 x 10^6 CFU/mL	100%	100%	100%
Leptospira interrogans	1 x 10^6 copies/mL	100%	100%	100%
Measles	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
MERS-Coronavirus	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Moraxella catarrhalis	1 x 10^6 CFU/mL	100%	100%	100%
Mumps	1 x 10^5 U/mL	100%	100%	100%
Mycobacterium tuberculosis Genomic DNA	1 x 10^6 copies/mL	100%	100%	100%
Mycoplasma pneumoniae	1 x 10^6 CCU/mL	100%	100%	100%
Neisseria elongata	1 x 10^6 CFU/mL	100%	100%	100%
Neisseria meningitidis	1 x 10^6 CFU/mL	100%	100%	100%
Parainfluenza Type 1	1 x 10^5 U/mL	100%	100%	100%
Parainfluenza Type 2	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza Type 3	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza Type 4	1 x 10^5 U/mL	100%	100%	100%
Parechovirus Type 3	1 x 10^5 U/mL	100%	100%	100%
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	100%	100%	100%
Rhinovirus 1A*	1 x 10^4 U/mL	95%	100%	100%
RSV-A	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
RSV-B	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Staphylococcus aureus	1 x 10^6 CFU/mL	100%	100%	100%

Staphylococcus epidermidis	1 x 10^6 CFU/mL	100%	100%	100%
Streptococcus pneumoniae	1 x 10^6 CFU/mL	100%	100%	100%
Streptococcus pyogenes	1 x 10^6 CFU/mL	100%	100%	100%
Streptococcus salivarius	1 x 10^6 CFU/mL	100%	100%	100%
Human Coronavirus RNA HKU1	1 x 10^5 U/mL	100%	100%	100%
Human Genomic DNA (Leukocytes)	1 x 10^6 cells/mL	100%	100%	100%
Pooled Human Nasal Wash	1:1 dilution	100%	100%	100%
SARS-COV1 Synthetic RNA	1 x 10^5 U/mL	100%	100%	100%

*A lower concentration was tested due to inability to obtain stock material with high titer
[1]CCU = Color Changing Units/milliliter
CFU/mL = Colony Forming Units/milliliter
IFU/mL = Infectious Units/milliliter
U/mL = Units/milliliter
$TCID_{50}$/mL = Tissue Culture Infectious Dose/milliliter

INTERFERING SUBSTANCES

Potentially interfering substances from respiratory specimens were tested for ability to generate false negative results. Samples were prepared by spiking each potentially interfering substance into a baseline sample consisting of pooled negative nasopharyngeal swab specimens and COVID-19 inactivated viral particles (2019-nCoV/USA-WA1/2020 strain), Influenza A/Michigan/45/2015 and Influenza B/Malaysia/2506/2004. The test samples contained each of the three viruses at a concentration of 1500 copies/mL (3X LOD). Testing was performed with three replicates per substance. The results are shown in Table 13. None of the substances tested interfere with the detection of COVID-19, influenza A or influenza B at the concentrations tested.

Table 13. Simplexa™ COVID-19 & Flu A/B Direct – Potentially Interfering Substances Results

Potentially Interfering Substance	Active Ingredient	Tested Concentration	COVID-19	Flu A	Flu B
Afrin Nasal spray	Oxymetazoline	15% (v/v)	100.0% (3/3	100.0% (3/3)	100.0% (3/3)
Antibacterial, systemic	Tobramycin	4 µg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Antibiotic, nasal ointment	Mupirocin	6.6 mg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3
Whole Blood	N/A	2% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3
Bovine submaxillary gland mucin, type I-S	Purified Mucin Protein	60 µg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Cold Eeze (Throat lozenges, Oral anesthetic and analgesic)	N/A	2.5% (w/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Nasal corticosteroid (Beconase AQ)	Beclomethasone	5% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Nasal corticosteroid (Flonase)	Fluticasone	5% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Relenza Antiviral Drug	Zanamivir	3.3 mg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Tamiflu Antiviral drug	Oseltamivir	1 µM	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Zicam Nasal Gel	*Luffa opperculata, Galphimia glauca*, histaminum hydrochloricum	5% (w/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Zicam Nasal Spray (Homeopathic allergy relief medicine)	N/A	10% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)

COMPETITIVE INTERFERENCE

Competitive Interference was performed to assess the ability of the assay to detect low doses of one (1) target analyte in the presence of high doses of another target analyte. Test specimens for wet testing were prepared by spiking one (1) target analyte at a low concentration (4X LOD) into negative matrix (NPS in UTM) in the presence of a high dose (1000X LOD) of one (1) of the other two (2) target analytes. All the possible target combinations were tested. Each contrived sample was tested in triplicate.
The results are shown in Table 14. All of the combinations tested showed no competitive interference for the detection of low concentrations of COVID-19, Flu A or Flu B in the presence of high concentrations of another assay target analyte.

Table 14. Simplexa™ COVID-19 & Flu A/B Direct – Competitive Interference Results

Low Concentration Target	High Concentration Target	Low Target Detection	High Target Detection
Flu A	N/A	100% (2/2)	N/A
	Flu B	100% (3/3)	100% (3/3)
	COVID-19	100% (3/3)	100% (3/3)
Flu B	N/A	100% (2/2)	N/A
	Flu A	100% (3/3)	100% (3/3)
	COVID-19	100% (3/3)	100% (3/3
COVID-19	N/A	100% (2/2)	N/A
	Flu A	100% (3/3)	100% (3/3)
	Flu B	100% (3/3)	100% (3/3)

N/A = not applicable

CARRY-OVER CONTAMINATION

Amplification carry-over for the Simplexa™ assays has been assessed against existing assays that use the same sample matrices, workflow and specimen type, and therefore no carry-over is anticipated. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was observed.

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GLOSSARY*

	Caution, consult accompanying documents.*		Telephone
	Consult instructions for use*		Fax
	Biological risk*	IVD	For *in vitro* diagnostic medical device*
	Contains sufficient for <n> tests*	REF	Catalog number*
	Temperature limitation*	REV	Revision
	Manufacturer*	LOT	Batch code*
	Use by*	REAG C	Direct Reaction Mix
	Do not reuse*	CONT	Kit Contents
	Keep away from sunlight*		

*ISO 15223-1

IFUK.CE.MOL4250
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