Simplexa™ COVID-19 Direct

REF MOL4150
Rev. 05
(English)

A real-time RT-PCR assay intended for the *in vitro* qualitative detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral RNA.

For Emergency Use Authorization Only
For *in vitro* diagnostic use
Rx Only

INTENDED USE

The DiaSorin Molecular Simplexa™ COVID-19 Direct real-time RT-PCR assay is intended for use on the LIAISON® MDX instrument for the *in vitro* qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS), nasal swabs (NS), nasal wash/aspirate (NW) or bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. The Simplexa™ COVID-19 Direct assay is an aid in the diagnosis of SARS-CoV-2 infection.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high or moderate complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and bronchoalveolar lavage (BAL) specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 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.

The Simplexa™ COVID-19 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. Simplexa™ COVID-19 Direct is only for use under the Food and Drug Administration's Emergency Use Authorization.

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.[5]

PRINCIPLES OF THE PROCEDURE

The DiaSorin Molecular Simplexa™ COVID-19 Direct assay system is a real-time RT-PCR system that enables the direct amplification of Coronavirus SARS-CoV-2 RNA from nasopharyngeal swabs (NPS), nasal swabs (NS), nasal wash/aspirate (NW) or bronchoalveolar lavage (BAL) specimens. The system consists of the Simplexa™ COVID-19 Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ COVID-19 Direct assay, fluorescent probes are used together with corresponding forward and reverse primers to amplify SARS-CoV-2 viral RNA and internal control RNA. The assay targets two different regions of the SARS-CoV-2 genome, ORF1ab and S gene. The S gene encodes the spike glycoprotein of the SARS-CoV-2 (COVID-19 virus) and is also targeted to specifically detect the presence of SARS-CoV-2. The ORF1ab region encodes well-conserved non-structural proteins and therefore is less susceptible to recombination. An RNA internal control is used to detect RT-PCR failure and/or inhibition.

MATERIALS PROVIDED

The Simplexa™ COVID-19 Direct assay 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 thirty (30) minutes of thawing.

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 Direct Reaction Mix	MOL4151	REAG	C	Co19	Brown	24	1/24	50 µL

COMPONENT DESCRIPTION

Kit Component	Contents				
Simplexa™ COVID-19 Direct Reaction Mix (RM)	DNA polymerase, Reverse transcriptase, RNase inhibitor, buffer, dNTPs, encapsulated RNA Template, fluorescent probes and corresponding forward and reverse primers specific for detection of SARS-CoV-2 viral RNA and for the Internal Control				
	Target	**Probe Fluorophore (Dye)**	**Excitation (nm)**	**Emission (nm)**	**Targeted Gene**
	S gene	FAM	495	520	S gene
	ORF1ab	JOE	520	548	ORF1ab
	Internal Control RNA (IC)	Q670	644	670	N/A
Simplexa™ COVID-19 Direct Barcode Card	Assay specific parameters and lot information.				

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 Positive Control Pack (REF MOL4160).
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).
5. Freezer (manual defrost) at -10 to -30 °C (for kit component and/or specimen frozen storage).
6. Refrigerator at 2 to 8 °C (for specimens).
7. Disposable, powder-free gloves.
8. Vortex for mixing patient samples.
9. Centrifuge for collecting contents to bottom of tubes.

RECOMMENDED MATERIALS

1. Universal Transport Media (UTM, Copan) or Universal Viral Transport (UVT, BD) 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 thirty (30) minutes.
5. Do not vortex the Reaction Mix.
6. Do not refreeze the Reaction Mix.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. For professional use only.
3. The Simplexa™ COVID-19 Direct real-time RT-PCR assay has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high or moderate complexity tests.
4. The Simplexa™ COVID-19 Direct real-time RT-PCR assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
5. The Simplexa™ COVID-19 Direct real-time RT-PCR assay is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of

COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

6. 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.
7. Do not pipette by mouth.
8. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
9. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
10. Treat all specimens and discs as capable of transmitting infectious agents.
11. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.[6,7]
12. 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.
13. Assay setup should be performed at room temperature (approximate range 18 to 25 °C).
14. Use calibrated fixed volume pipettes or equivalent to transfer sample and Reaction Mix.
15. Avoid touching the underside of the foil that will be in contact with the wells and disc surface.
16. To prevent potentially erroneous results, make sure that the sample and reagent are added to the appropriate input wells.
17. 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.
18. Initiate the run within thirty (30) minutes of removing the Reaction Mix vial from the freezer.
19. 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.
20. Discs may be reused until all eight (8) wedges have been used. Dispose of used discs without detaching foil cover in biohazardous waste container.
21. After each use store Direct Amplification Disc flat with the numbered foil side up.
22. Store reagents away from light.
23. Reaction Mix contains > 1% glycerol. Upon inhalation or skin contact, first aid measures should be taken.
24. If kit packaging or contents appear to be broken or damaged do not use and contact DiaSorin Molecular. Contact information is on the last page of this document.
25. 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 Technical Services. The contact information is on the last page of this document.
26. Not installing or changing the spectral matrix can result in false results.

INSTRUCTIONS FOR USE

A. SPECIMEN COLLECTION AND HANDLING

Acceptable specimen types include:

- Nasopharyngeal swabs (NPS) or nasal swabs (NS) in Copan Universal Transport Media (UTM) or BD Universal Viral Transport (UVT) or equivalent, Remel M5, Remel M6, Copan ESwab™ (Liquid Amies), Puritan® UniTranz-RT®, or saline (0.9% sodium chloride in water). Use only 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.
- Bronchoalveolar lavage (BAL) undiluted or diluted 1:1 (v/v) in a mucolytic such as Remel Sputasol.
- Nasal wash/aspirate (NW) undiluted.

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.

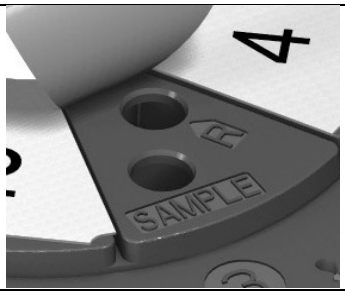
C. DIRECT AMPLIFICATION DISC LOADING AND REAL-TIME PCR AMPLIFICATION

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 (1) Reaction Mix vial for each sample or control to be tested.
3. Scan the barcode on the Simplexa™ COVID-19 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 Sample (SAMPLE) and Reaction (R) 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. Use the fixed volume pipette to transfer 50 µL of samples 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. If the original foil is torn do not load the wells in the wedge. Instead load another 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 DAD 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 Positive Control Pack (MOL4160) may be used as an external control for Quality Control (QC) testing, training or proficiency testing. Each laboratory should establish its own QC ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice. Refer to the Simplexa™ COVID-19 Positive Control Pack (IFUC.US.MOL4160) for instructions on running the positive control.

Expected Quality Control Results

Control Type	ORF1ab target	S gene target	RNA Internal Control (RNA IC)
Simplexa™ COVID-19 Positive Control[1]	Positive	Positive	Not applicable[2]
No Template Control (NTC)	Negative	Negative	Valid

[1]Typical Ct values for the Positive Control range between 22 to 32.
[2]Detection of the Simplexa™ RNA Internal Control (RNA IC) is not required for a valid result when SARS-CoV-2 is detected.

INTERPRETATION OF RESULTS

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

1. For each accession ID (Sample ID) entered, the software displays a result ("Positive", "Negative", "Invalid", "EC500, EC505 or EC515") for SARS-CoV-2 RNA.

Results		**Interpretation**
SARS-Cov-2 Target		
ORF1ab	**S gene**	
Positive	**Positive**	Result indicates the presence of SARS-CoV-2 RNA in the patient sample.
Positive	**----**	Result indicates the presence of SARS-CoV-2 RNA in the patient sample.*
----	**Positive**	Result indicates the presence of SARS-CoV-2 RNA in the patient sample.*
Negative	**Negative**	Result indicates the absence of SARS-CoV-2 RNA in the patient sample.
Invalid		Result indicates inability to conclusively determine presence or absence of SARS-CoV-2 RNA 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.

Results	Interpretation
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.

* In the case of one SARS-CoV-2 target positive/one SARS-CoV-2 target negative, result is suggestive of: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in one of the target regions, or 3) other factors.

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

INVALID RESULTS

In case of an "Invalid" result, re-test the sample with a new Reaction Mix vial from the same kit or a new kit. If the problem is unresolved, contact the DiaSorin Molecular Technical Services department. Contact information can be found on the last page of this document.

LIMITATIONS

1. For Emergency Use Authorization Only use only.
2. For *in vitro* diagnostic use.
3. For professional use only.
4. Testing of nasal swabs even if collected by a healthcare provider is limited to patients with symptoms of COVID-19.
5. Not for screening.
6. 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.
7. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
8. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
9. Information on the kit barcode can only be transferred into the LIAISON® MDX Studio Software Studio 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.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The Simplexa™ COVID-19 Direct Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/ EmergencySituations/ucm161496.htm.

However, to assist clinical laboratories using the Simplexa™ COVID-19 Direct, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using Simplexa™ COVID-19 Direct will include with result reports of Simplexa™ COVID-19, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using Simplexa™ COVID-19 Direct will use Simplexa™ COVID-19 Direct as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use Simplexa™ COVID-19 Direct are not permitted.

C. Authorized laboratories that receive Simplexa™ COVID-19 Direct will notify the relevant public health authorities of their intent to run Simplexa™ COVID-19 Direct prior to initiating testing.

D. Authorized laboratories using Simplexa™ COVID-19 Direct will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of Simplexa™ COVID-19 Direct and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and DiaSorin Molecular (via phone: (800) 838-4548) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of Simplexa™ COVID-19 Direct of which they become aware.

F. All laboratory personnel using Simplexa™ COVID-19 Direct must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use Simplexa™ COVID-19 Direct in accordance with the authorized labeling.

[1] The letter of authorization refers to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high and moderate complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

CLINICAL EVALUATION

The clinical performance of the Simplexa™ COVID-19 Direct assays was established in multi-site clinical evaluation. Fresh clinical NPS specimens were tested with the Simplexa™ COVID-19 Direct assay at three (3) different clinical sites from February 2020 to March 2020. For each of the sites, an established comparator was used. Sites 1 and 3 used the same comparator while Site 2 used a different comparator. Negative individually collected and positive contrived NS or NW specimens were tested internally with the Simplexa™ COVID-19 Direct assay in April or May 2020. Contrived samples were prepared by spiking heat-inactivated 2019-nCoV/USA-WA1/2020 strain (ATCC® VR-1986HK™) into individual negative NS or NW specimens. BAL specimens, diluted 1:1 with Sputasol, were tested with the Simplexa™ COVID-19 Direct assay at one clinical site using an established comparator.

Simplexa™ COVID-19 Direct			Comparator		Agreement*/**
			Positive (+)	Negative (-)	N/N (%)
NPS	Site 1	Positive (+)	1	0	**PPA:** 1/1 (100%)
		Negative (-)	0	20	**NPA:** 20/20 (100%)
		Site Total	**1**	**20**	
	Site 2	Positive (+)	11	0	**PPA:** 11/11 (100%)
		Negative (-)	0	0	**NPA:** 0/0 (100%)
		Site Total	**11**	**0**	
	Site 3	Positive (+)	40	0	**PPA:** 40/40 (100%)
		Negative (-)	0	36	**NPA**: 36/36 (100%)
		Site Total	**40**	**36**	
NS	Site 4	Positive (+)	30	0	**PPA:** 30/30 (100%)
		Negative (-)	0	30	**NPA**: 30/30 (100%)
		Site Total	**30**	**30**	
NW	Site 4	Positive (+)	29	0	**PPA:** 29/30 (96.7%)
		Negative (-)	1	30	**NPA**: 30/30 (100%)
		Site Total	30	30	
BAL	Site 1	Positive (+)	11	0	**PPA:** 11/11 (100%)
		Negative (-)	0	7	**NPA**: 7/7 (100%)
		Site Total	**11**	**7**	

*NPA = Negative Percent Agreement, PPA = Positive Percent Agreement
**NS and NW agreement (PPA and NPA) was determined against expected results.

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) for NPS was determined to be the lowest detectable concentration of quantitated extracted viral genomic RNA (copies/mL) at which ≥ 95% of all replicates test positive. Initially, the tentative LoD was identified with serial dilutions of the characterized SARS-CoV-2 viral genomic RNA 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 forty eight (48) 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.

The final LoD for NPS, according to the assay results interpretation, is 500 copies/mL.

Analytical Sensitivity/Limit of Detection for Viral Genomic RNA in UTM with RNasin

COVID-19 genomic RNA Copies/mL	Interpretation	S gene (FAM)		ORF1ab (JOE)	
		% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)	% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)
2000	100% (48/48) Positive	100% (48/48)	31.0 ± 0.64 (2.1%)	100% (48/48)	31.3 ± 0.74 (2.4%)
1000	100% (48/48) Positive	95.8% (46/48)	32.4 ± 1.01 (3.1%)	93.8% (45/48)	32.7 ± 1.08 (3.3%)
500	100% (48/48) Positive	95.8% (46/48)	33.4 ± 1.31 (3.9%)	70.8% (34/48)	33.9 ± 0.98 (2.9%)

The Limit of Detection (LoD) for NS was determined to be the lowest detectable concentration of inactivated titered COVID-19 viral particles, strain 2019-nCoV/USA-WA1/2020, at which ≥ 95% of all replicates tested positive according to the results interpretation algorithm in pooled negative nasal swab specimens in UTM. Initially, the tentative LoD was identified with serial dilutions of the viral particles tested in four (4) replicates. 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.

The final LoD for NS, according to the assay results interpretation, is 242 copies/mL.

Analytical Sensitivity/Limit of Detection for Inactivated Viral Particles in NS matrix in UTM

COVID-19 Genome Copies / mL	Interpretation	S gene (FAM)		ORF1ab (JOE)	
		% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)	% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)
242	100% (20/20) Positive	80% (16/20)	34.0 ± 0.83 (2.4%)	80% (16/20)	32.9 ± 0.75 (2.3%)

The Limit of Detection (LoD) for NW was determined to be the lowest detectable concentration of inactivated titered COVID-19 viral particles, strain 2019-nCoV/USA-WA1/2020, at which ≥ 95% of all replicates tested positive in pooled negative NW specimen matrix. Initially, the tentative LoD was identified with serial dilutions of the viral particles tested in four (4) replicates. 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.

The final LoD for NW, according to the assay results interpretation, is 500 copies/mL.

Analytical Sensitivity/Limit of Detection for Inactivated Viral Particles in Nasal Wash/Aspirate matrix

COVID-19 Genome Copies / mL	Interpretation	S gene (FAM)		ORF1ab (JOE)	
		% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)	% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)
500	100% (20/20) Positive	85.0% (17/20)	33.1 ± 1.01 (3.0%)	95.0% (19/20)	32.3 ± 0.89 (2.8%)

The Limit of Detection (LoD) for BAL was determined to be the lowest detectable concentration of inactivated titered COVID-19 viral particles, strain 2019-nCoV/USA-WA1/2020, at which ≥ 95% of all replicates tested positive in pooled negative BAL matrix. Initially, the tentative LoD was identified with serial dilutions of the viral particles tested in four (4) replicates. 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.

The final LoD for BAL, according to the assay results interpretation, is 1208 copies/mL.

Analytical Sensitivity/Limit of Detection for Inactivated Viral Particles in BAL matrix

COVID-19 Genome Copies / mL	Interpretation	S gene (FAM)		ORF1ab (JOE)	
		% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)	% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)
1208	100% (20/20) Positive	90.0% (18/20)	32.8 ± 0.99 (3.0%)	95% (19/20)	32.2 ± 0.96 (3.0%)

REACTIVITY/INCLUSIVITY

An *in silico* inclusivity analysis of the Simplexa™ COVID-19 Direct primers and probes was performed. All primer sets designed for detection of the ORF1ab and S gene were tested against the complete available SARS-CoV-2 genome sequence. The analysis demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available SARS-CoV-2 sequences from the National Center for Biotechnology Information (NCBI) and Global Initiative on Sharing Avian Influenza Data (GISAID) databases/databanks.

Database	Identity to ORF1ab		Identity to S gene	
	Primers (%)	Probe (%)	Primers (%)	Probe (%)
NCBI	52/52 (100%)	52/52 (100%)	53/53 (100%)	53/53 (100%)

GISAID	352/352 (100%)	350/352 (99%)	364/364 (100%)	364/364 (100%)

CROSS REACTIVITY

Cross-reactivity of the Simplexa™ COVID-19 Direct assay was evaluated using both *in silico* analysis and by testing whole organisms or purified nucleic acid from other organisms. Test specimens for laboratory testing were prepared by spiking cultured isolates/inactivated organisms/purified nucleic acids (whole genome) (i.e., 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 (UTM) and determining cross reactivity based on three replicates. RNasin® was added to UTM for specimens containing extracted RNA. The results from the cross-reactivity, both *in silico* and wet testing, are summarized below.

***In silico* Cross Reactivity Analysis**

Microorganism	***In silico* Analysis for % Identity target: ORF1ab**	***In silico* Analysis for % Identity target: S gene**
Human coronavirus 229E	No alignment found	No alignment found
Human coronavirus OC43	No alignment found	No alignment found
Human coronavirus HKU1	No alignment found	No alignment found
Human coronavirus NL63	No alignment found	No alignment found
SARS-coronavirus*	90%	80%
MERS-coronavirus	No alignment found	No alignment found
Adenovirus C	No alignment found	No alignment found
Human Metapneumovirus (hMPV)	No alignment found	No alignment found
Parainfluenza virus 1	No alignment found	No alignment found
Parainfluenza virus 2	No alignment found	No alignment found
Parainfluenza virus 3	No alignment found	No alignment found
Parainfluenza virus 4	No alignment found	No alignment found
Influenza A	No alignment found	No alignment found
Influenza B	No alignment found	No alignment found
Enterovirus (e.g. EV68)	No alignment found	No alignment found
Respiratory Syncytial Virus	No alignment found	No alignment found
Rhinovirus	No alignment found	No alignment found
Chlamydia pneumonia	No alignment found	No alignment found
Haemophilus influenzae	No alignment found	No alignment found
Mycobacterium tuberculosis	No alignment found	No alignment found
Streptococcus pneumonia	No alignment found	No alignment found
Streptococcus pyogenes	No alignment found	No alignment found
Bordetella pertussis	No alignment found	No alignment found
Mycoplasma pneumoniae	No alignment found	No alignment found
Pneumocystis jirovecii (PJP)	No alignment found	No alignment found
Influenza C	No alignment found	No alignment found
Parechovirus	No alignment found	No alignment found
Candida albicans	No alignment found	No alignment found
Corynebacterium diphtheriae	No alignment found	No alignment found
Legionella pneumophila	No alignment found	No alignment found
Legionella non-pneumophila	No alignment found	No alignment found
Bacillus anthracis (Anthrax)	No alignment found	No alignment found
Moraxella catarrhalis	No alignment found	No alignment found

Microorganism	*In silico* Analysis for % Identity target: ORF1ab	*In silico* Analysis for % Identity target: S gene
Neisseria elongate	No alignment found	No alignment found
Neisseria meningitidis	No alignment found	No alignment found
Pseudomonas aeruginosa	No alignment found	No alignment found
Staphylococcus epidermis	No alignment found	No alignment found
Streptococcus salivarius	No alignment found	No alignment found
Leptospirosis	No alignment found	No alignment found
Chlamydia psittaci	No alignment found	No alignment found
Coxiella burnetii (Q-Fever)	No alignment found	No alignment found

* The percent (%) homology indicated in this table is for the target gene. Considering the 55% or less homology between primers and ORF1ab and S gene in SARS Coronavirus, amplification is not possible.

Laboratory Tested Cross Reactivity Analysis

Organism	Qualitative Results: % Detection (# Detected/#Tested)		
	S gene (FAM)	ORF1ab (JOE)	IC(Q670)
Adenovirus 1	0% (0/3)	0% (0/3)	100% (3/3)
Bordetella pertussis	0% (0/3)	0% (0/3)	100% (3/3)
Chlamydophila pneumoniae	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus 229E	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus HKU1*	N/A	N/A	N/A
Coronavirus NL63	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus OC43	0% (0/3)	0% (0/3)	100% (3/3)
Enterovirus 68	0% (0/3)	0% (0/3)	100% (3/3)
Haemophilus influenzae	0% (0/3)	0% (0/3)	100% (3/3)
Human metapneumovirus (hMPV-9)	0% (0/3)	0% (0/3)	100% (3/3)
Human leukocytes (human genomic DNA)	0% (0/3)	0% (0/3)	100% (3/3)
Influenza A H3N2 Hong Kong/8/68	0% (0/3)	0% (0/3)	100% (3/3)
Influenza B/Phuket/3073/2013	0% (0/3)	0% (0/3)	100% (3/3)
Legionella pneumophila	0% (0/3)	0% (0/3)	100% (3/3)
MERS-Coronavirus (Extracted RNA)	0% (0/3)	0% (0/3)	100% (3/3)
Mycobacterium tuberculosis (genomic DNA)	0% (0/3)	0% (0/3)	100% (3/3)
Mycoplasma pneumoniae	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Type 1	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Type 2	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Type 3	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Type 4A	0% (0/3)	0% (0/3)	100% (3/3)
Pooled Human Nasal Fluid	0% (0/3)	0% (0/3)	100% (3/3)
Rhinovirus B14	0% (0/3)	0% (0/3)	100% (3/3)
RSV A Long	0% (0/3)	0% (0/3)	100% (3/3)
RSV B Washington	0% (0/3)	0% (0/3)	100% (3/3)
SARS-Coronavirus (Purified RNA)	0% (0/3)	0% (0/3)	100% (3/3)
SARS-Coronavirus HKU39849 (Extracted RNA)	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus pneumoniae	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus pyogenes	0% (0/3)	0% (0/3)	100% (3/3)

* Coronavirus HKU1 was not available for testing; however, this organism was evaluated *in silico*. No alignments with Simplexa™ COVID-19 Direct primers and probes were found

POTENTIAL INTERFERING SUBSTANCES

Potential interfering substances from respiratory specimens were tested for ability to generate false negative results using samples containing the extracted viral RNA at 3x LoD in nuclease free water. Testing was performed with 3 replicates per substance.

Potential Interfering Substance	Active Ingredient	Tested Concentration	COVID-19 Qualitative % Detection (# Detected / #Tested)	IC Qualitative % Detection (# Detected / #Tested)
Systemic antibacterial	Tobramycin	4 µg/mL	100% (3/3)	100% (3/3)
Antibiotic nasal ointment	Mupirocin	6.6 mg/mL	100% (3/3)	100% (3/3)
Nasal corticosteroids	Fluticasone	5% (v/v)	100% (3/3)	100% (3/3)
Nasal gel	Luffa Opperculata, Galphimia glauca, histaminum hydrochloricum	5% (w/v)	100% (3/3)	100% (3/3)
Homeopathic allergy relief medicine	Not Applicable	10% (v/v)	100% (3/3)	100% (3/3)
Nasal spray or drops	Oxymetazoline	15% (v/v)	100% (3/3)	100% (3/3)
Cold Eeze (Throat lozenges, Oral anesthetic and analgesic)	Not Applicable	2.5% (w/v)	100% (3/3)	100% (3/3)
Anti-viral drug	Oseltamivir	3.3 mg/mL	100% (3/3)	100% (3/3)
Bovine submaxillary gland mucin, type I-S	Mucin	60 µg/mL	100% (3/3)	100% (3/3)
Whole Blood	Not Applicable	2%(v/v)	100% (3/3)	100% (3/3)

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GLOSSARY

	Caution, consult accompanying documents*		Telephone
	Consult instructions for use*		Fax
Rx Only	Prescription only	**IVD**	*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

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