DiaSorin Molecular LLC
Tara Viviani
Director, Regulatory Affairs
11331 Valley View Street
Cypress, California 90630

September 13, 2022

Re: K212147
Trade/Device Name: Simplexa COVID-19 Direct
Regulation Number: 21 CFR 866.3981
Regulation Name: Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test
Regulatory Class: Class II
Product Code: QQX
Dated: August 12, 2022
Received: August 15, 2022

Dear Tara Viviani:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Himani Bisht, Ph.D.
Assistant Director
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K212147

Device Name
Simplexa™ COVID-19 Direct

Indications for Use *(Describe)*

The DiaSorin Molecular Simplexa™ COVID-19 Direct is real-time RT-PCR assay 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) and nasal swabs (NS) from symptomatic 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.

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 co-infection with other pathogens. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.

Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Applicant	DiaSorin Molecular LLC. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Tara Viviani Senior Director, Molecular Regulatory Affairs Tel. 562.240.6271 Tara.Viviani@DiaSorin.com
Summary Date	September 13, 2022
Proprietary Name	Simplexa™ COVID-19 Direct
US Product Codes/Names and Regulation Numbers	QQX- Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test (21 CFR § 866.3981) OOI - Instrumentation for clinical multiplex test systems (21 CFR § 862.2570)
Classification	Class II
Predicate Devices	BioFire COVID-19 Test 2 (K211079)

Intended Use

Simplexa™ COVID-19 Direct REF MOL4150

The DiaSorin Molecular Simplexa™ COVID-19 Direct is a real-time RT-PCR assay 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) and nasal swab specimens from symptomatic 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.

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 co-infection with other pathogens. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.

Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.

Device Description

The Simplexa COVID-19 Direct is a real-time RT-PCR (rRT-PCR) system that enables the direct amplification and detection of SARS-CoV-2 (COVID-19) RNA from nasopharyngeal swab or nasal swab that has not undergone nucleic acid extraction. The system consists of the Simplexa COVID-19 Direct reaction mix, the LIAISON MDX (with LIAISON MDX Studio Software), the Direct Amplification Disc and

associated accessories. The assay uses forward and reverse primers and associated fluorescent probe(s) included in the reaction mix to amplify SARS-CoV-2 cDNA reverse transcribed from RNA. The primers and probe sets are designed to detect SARS-CoV-2 ORF1ab and S gene from the viral RNA in nasopharyngeal swab or nasal swab. An RNA internal control, with associated primers and a fluorescent probe, is included in the reaction mix to detect RT-PCR failure and/or inhibition.

Simplexa™ COVID-19 Direct REF MOL4150

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

Simplexa™ COVID-19 Direct Components and Descriptions

<table>
<tr><th>Kit Component</th><th colspan="5">Contents</th></tr>
<tr><td rowspan="5">Simplexa™ COVID-19 Direct Reaction Mix (RM)</td><td colspan="5">DNA polymerase, reverse transcriptase, RNase inhibitor, buffer, dNTPs, encapsulated RNA template (Internal Control), fluorescent probes and corresponding forward and reverse primers specific for detection of SARS-CoV-2 RNA and for the RNA Internal Control.</td></tr>
<tr><td>Target</td><td>Probe Fluorophore (Dye)</td><td>Excitation (nm)</td><td>Emission (nm)</td><td>Targeted Gene</td></tr>
<tr><td>S gene</td><td>FAM</td><td>445-505</td><td>507-533</td><td>S gene</td></tr>
<tr><td>ORF1ab</td><td>JOE</td><td>503-543</td><td>547-573</td><td>ORF1ab</td></tr>
<tr><td>Internal Control RNA (IC)</td><td>Q670</td><td>622-658</td><td>652-708</td><td>N/A</td></tr>
<tr><td>Simplexa™ COVID-19 Direct Barcode</td><td colspan="5">Assay specific parameters and lot information</td></tr>
</table>

Materials Supplied Separately

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

Materials Required But Not Supplied

LIAISON® MDX with LIAISON® MDX Studio Software version 1.1 or higher.
Simplexa™ COVID-19 Positive Control Gen II Pack (REF MOL4165).
50 µL fixed volume pipette (VWR Signature™ Fixed Volume Ergonomic High-Performance Pipettor Model VWR FE50 or equivalent).
Sterile, nuclease-free disposable pipette tips with filters (Extra Long tips ≥ 91 mm are recommended for pipetting directly from primary collection tubes).
Freezer (manual defrost) at -10 to -30 °C (for kit component and/or specimen frozen storage).
Refrigerator at 2 to 8 °C (for specimens).
Disposable, powder-free gloves.
Vortex for mixing patient samples.
Centrifuge for collecting contents to bottom of tubes.

Comparison to Predicate Device

Comparison to Predicate Device	Predicate Device: BioFire COVID-19 Test 2 (K211079)	Candidate Device: Simplexa COVID-19 Direct Simplex COVID-19 Positive Control Gen II Pack
Product Code	QQX	Same
Regulation Number	21 CFR 866.3981	Same
Organism Detected	SARS-CoV-2	Same
Measurand	RNA from SARS-CoV-2	Same
Intended Use Kit	The BioFire COVID-19 Test 2 is a qualitative nested multiplexed RT-PCR in vitro diagnostic test intended for use with the BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire COVID-19 Test 2 detects nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS) from symptomatic individuals suspected of COVID-19 by their healthcare provider. Results are for the identification of SARS-Co V-2 RNA. The SARS-Co V-2 RNA is generally detectable in NPS 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 co-infection with other pathogens. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire COVID-19 Test 2 is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.	Simplexa™ COVID-19 Direct REF MOL4150 The DiaSorin Molecular Simplexa™ COVID-19 Direct is a real-time RT-PCR assay 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) and nasal swabs (NS) from symptomatic 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. 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 co-infection with other pathogens. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.
Automated System (Sample to Answer)	Automated	Same
Instrumentation	BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems	LIAISON MDX
Sample Types	Nasopharyngeal Swab	Nasopharyngeal Swab, Nasal Swab

CLINICAL AGREEMENT

The clinical performance of the Simplexa™ COVID-19 Direct was established in multi-site clinical evaluation. A total of 1,150 prospective fresh and/or prospective frozen Nasopharyngeal (NPS) and/or

Nasal Swabs (NS) samples were collected from four (4) geographically diverse collection sites, one of which was outside the United States (OUS). A total of 409 NPS samples and a total of 741 nasal swabs were collected. One hundred fourteen (114) samples were excluded from the analysis due to insufficient evidence to include certain media types, 24 were excluded due to invalid results, and one (1) specimen was excluded due to an indeterminate result from the composite reference method. The Simplexa™ COVID-19 Direct Clinical Agreement testing was performed at three (3) external clinical sites and one (1) internal site from October 2020 to April 2021. Three COVID-19 EUA approved NAAT assays were used to establish a comparator reference method (CRM) to evaluate the performance of the candidate device. Two out of three positive results determined "Detected" CRM and two out of three negative results determined "Not Detected" CRM. The clinical performance of Simplexa™ COVID-19 Direct is summarized in the Tables 1-3 below.

Table 1: Total Specimen Clinical Summary: Simplexa™ COVID-19 Direct vs. EUA NAAT CRM

Simplexa™ COVID-19 Direct Prospective Upper Respiratory	**CRM**		
	Detected	**Not Detected**	**Total**
Detected	108	4	112
Not Detected	2	897	899
Total	110	901	1011
PPA:	98.2% (108/110) 95% CI: 93.6% to 99.5%		
NPA:	99.6% (897/901) 95% CI: 98.9% to 99.8%		

Table 2: NPS Clinical Summary: Simplexa™ COVID-19 Direct vs. EUA NAAT CRM

Simplexa™ COVID-19 Direct Prospective NPS	**CRM**		
	Detected	**Not Detected**	**Total**
Detected	60	1[a]	61
Not Detected	1	237	238
Total	61	238	299
PPA:	98.4% (60/61) 95% CI: 91.3% to 99.7%		
NPA:	99.6% (237/238) 95% CI: 97.7% to 99.9%		

[a] One sample positive by Simplexa was detected one of the NAATs included in the CRM.
CRM = Composite Reference Method, PPA = Percent Positive Agreement, NPA = Negative Percent Agreement

Table 3: NS Collection: Simplexa™ COVID-19 Direct vs. EUA NAAT CRM

Simplexa™ COVID-19 Direct Prospective NS	CRM		
	Detected	Not Detected	Total
Detected	48	3[a]	51
Not Detected	1	660	661
Total	49	663	712
PPA:	98.0% (48/49) 95% CI: 89.3% to 99.6%		
NPA:	99.5% (660/663) 95% CI: 98.7% to 99.8%		

[a] One sample positive by Simplexa was positive by one of the NAATs in the CRM.
CRM = Composite Reference Method, PPA = Percent Positive Agreement, NPA = Negative Percent Agreement

REPRODUCIBILITY

The Simplexa™ COVID-19 Direct reproducibility study was performed at two (2) external clinical sites and one (1) internal site to evaluate repeatability, between day, between operator, between site and total reproducibility of the assay. The panel consisted of a total of four (4) panel members including two (2) contrived panel samples, one (1) positive control and one (1) universal transport media (UTM) negative sample. The contrived panel members were prepared at approximately one to two times (1x to 2x) the Limit of Detection (LoD) for a low positive (LP) and three to five times (3x to 5x) LoD for a moderate positive (MP). Both contrived panel members were built with SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020 spiked into native negative nasopharyngeal swab matrix. Each panel member was tested in triplicate per run for two (2) runs per day for a total of five (5) non-consecutive testing days at three (3) testing sites for a total of ninety (90) replicates. Each site had two (2) operators who each assayed the entire panel once per day, for a total of two (2) sets of data per day. The quantitative summary of variance components for each panel member based on the algorithm is in Table 4 below.

Table 4. Simplexa™ COVID-19 Direct Reproducibility

Sample		Site 1		Site 2		Site 4		All Sites	
		Agree-ment with Expected Results	Avg. Ct ± SD (%CV)	Agree-ment with Expected Results	Avg. Ct ± SD (%CV)	Agree-ment with Expected Results	Avg. Ct ± SD (%CV)	Agree-ment with Expected Results	95% CI
NPS_WA-1_LP	S gene	93.3% (28/30)	31.6 ± 0.95 (3.0%)	90.0% (27/30)	32.4 ± 1.14 (3.5%)	100.0% (30/30)	31.6 ± 0.72 (2.3%)	94.4% (85/90)	88% - 98%
	ORF1ab	90.0% (27/30)	32.2 ± 0.97 (3.0%)	96.7% (29/30)	32.7 ± 1.18 (3.6%)	100.0% (30/30)	31.9 ± 1.09 (3.4%)	95.6% (86/90)	89% - 98%
	Total[a]	100.0% (30/30)	N/A	96.7% (29/30)	N/A	100.0% (30/30)	N/A	98.9% (89/90)	94% - 100%
NPS_WA-1_MP	S gene	100.0% (30/30)	30.5 ± 0.80 (2.6%)	90.0% (27/30)	30.8 ± 1.11 (3.6%)	96.7% (29/30)	31.1 ± 1.14 (3.7%)	95.6% (86/90)	89% - 98%

Sample		Site 1		Site 2		Site 4		All Sites	
		Agreement with Expected Results	Avg. Ct ± SD (%CV)	Agreement with Expected Results	Avg. Ct ± SD (%CV)	Agreement with Expected Results	Avg. Ct ± SD (%CV)	Agreement with Expected Results	95% CI
	ORF1ab	100.0% (30/30)	31.3 ± 0.87 (2.8%)	100.0% (30/30)	31.6 ± 1.39 (4.4%)	100.0% (30/30)	30.7 ± 0.98 (3.2%)	100.0% (90/90)	96% - 100%
	Total[a]	100.0% (30/30)	N/A	100.0% (30/30)	N/A	100.0% (30/30)	N/A	100.0% (90/90)	96% - 100%
Negative (UTM)	S gene	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (90/90)	96% - 100%
	ORF1ab	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (90/90)	96% - 100%
	Total[a]	100.0% (30/30)	N/A	100.0% (30/30)	N/A	100.0% (30/30)	N/A	100.0% (90/90)	96% - 100%
Positive Control (PC)	S gene	100.0% (30/30)	25.8 ± 0.26 (1.0%)	100.0% (30/30)	25.7 ± 0.18 (0.7%)	100.0% (30/30)	25.7 ± 0.42 (1.6%)	100.0% (90/90)	96% - 100%
	ORF1ab	100.0% (30/30)	26.3 ± 0.32 (1.2%)	100.0% (30/30)	26.0 ± 0.12 (0.5%)	100.0% (30/30)	25.8 ± 0.36 (1.4%)	100.0% (90/90)	96% - 100%
	Total[a]	100.0% (30/30)	N/A	100.0% (30/30)	N/A	100.0% (30/30)	N/A	100.0% (90/90)	96% - 100%
Total	S gene	98.3% (118/120)		95.0% (114/120)		99.2% (119/120)		97.5% (351/360)	95% - 99%
	ORF1ab	97.5% (117/120)		99.2% (119/120)		100.0% (120/120)		98.9% (356/360)	97% - 100%
	Total[a]	100.0% (120/120)		99.2% (119/120)		100.0% (120/120)		99.7% (359/360)	98% - 100%

[a]Total based on algorithm that at least one target (S gene or ORF1ab) is Detected for the sample to be Detected for SARS-CoV-2.

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) for NPS and NS was determined to be the lowest detectable concentration of inactivated viral particles (copies/mL) for strain 2019-nCoV/USA-WA1/2020 at which ≥ 95% of all replicates tested positive. Initially, the tentative LoD was identified with serial dilutions of the inactivated viral particles in five (5) replicates for NPS matrix in UTM or four (4) replicates for NS matrix in UTM. The lowest concentration at which all replicates were positive was interpreted as the tentative LoD. For NPS, the LoD was then confirmed by testing forty (40) replicates with concentrations at the tentative LoD while for NS, the LoD was confirmed by testing twenty (20) replicates with concentrations at the tentative LoD. For both NPS and NS, the final LoD was confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity. Table 5 shows the results of the studies.

The final LoD for NPS, according to the assay results interpretation was 500 copies/mL. The LoD for NS, according to the assay results interpretation was 242 copies/mL.

Table 5: Analytical Sensitivity/Limit of Detection for Inactivated Viral Particles in NPS or NS Matrix in UTM

Specimen Type	SARS-CoV-2 LoD (copies/mL)	% Detection (# Detected / # Tested)	S gene (FAM)		ORF1ab (JOE)	
			% Detection (# Detected/# Tested)	Mean Ct ± SD (%CV)	% Detection (# Detected/# Tested)	Mean Ct ± SD (%CV)
NPS	500	100% (40/40) [a]	90% (36/40)	32.5 ± 1.60 (4.9%)	100% (40/40)	31.6 ± 1.56 (4.9%)
NS	242	100% (20/20) [a]	80% (16/20)	34.0 + 0.83 (2.4%)	80% (16/20)	32.9 + 0.75 (2.3%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation

[a] % Detection based on algorithm that at least one target (S gene or ORF1ab) is Detected for the sample to be Detected for SARS-CoV-2.

The Limit of Detection (LoD) using inactivated SARS-CoV-2 WHO International Standard viral particles in NPS matrix was determined. The tentative LoD was identified with serial dilutions of the viral particles. The final LoD was confirmed to be the lowest concentration quantified in International Units per milliliter (IU/mL) resulting in positive detection with a minimum 95% positivity. Table 6 shows the results of the study.

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

Table 6: Analytical Sensitivity/Limit of Detection for SARS-CoV-2 WHO International Standard

SARS-CoV-2 WHO International Standard (IU/mL)	% Detection (# Detected / # Tested)	S gene (FAM)		ORF1ab (JOE)	
		% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)	% Detection (# Detected / # Tested)	Mean Ct ± SD (%CV)
500	97.5% (39/40)	95% (38/40)	33.2 ± 1.28 (3.8%)	85% (34/40)	33.6 ± 1.36 (4.1%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation, IU = International Units

FDA SARS-CoV-2 Reference Panel Testing: The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), testing blinded samples and according to a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for the Limit of Detection (LoD). Blinded sample testing was used to establish specificity and to confirm the LoD. The results are summarized in Table 7.

Table 7: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NPS	6×10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL, N/A = Not applicable, ND = Not detected, NPS = Nasopharyngeal Swab

REACTIVITY/INCLUSIVITY

In Silico

An *in silico* inclusivity analysis of the COVID-19 target primers and probes in the Simplexa™ COVID-19 Direct assay was performed. All primer and probe sets designed for detection of the ORF1ab and S gene were tested against the complete SARS-CoV-2 genome sequences available in the GISAID database submitted from November 01, 2021 to January 31, 2022. The analysis included 2,170,584 sequences in the amplicon regions of the ORF1ab and S gene primer/probe regions. Only target sequences with full coverage of all three ORF1ab and S gene forward and reverse primer as well as probe region were included in the analyses. The analysis showed that the Simplexa™ COVID-19 Direct target regions had no mismatch to 2,170,382 sequences (~99.99%) and were predicted to be detected by the assay based on sequence homology. There were 202 sequences (~0.01%) with mismatches in at least one primer or probe binding region, region in either ORF1ab or S gene target region. A melting temperature (Tm) analysis was conducted for those sequences with mismatches in the binding sites of both gene assay target regions. A Tm calculation was performed with assay-specific conditions using a Tm Mismatch Bioinformatics Tool. Tm values observed above their respective annealing temperature had mismatches that were not located at the 3' end for the primers; as such, detection of these sequences is not affected by the mismatches.

An additional in silico inclusivity analysis was performed for complete SARS-CoV-2 genome sequences available in the GISAID database submitted from February 01, 2022 to April 30, 2022 including sequences of the Omicron BA.4 and BA.5 subvariants. The analysis included 377,668 sequences in the amplicon regions of the ORF1ab and S gene primer/probe regions. Only target sequences with full coverage of all three ORF1ab and S gene forward and reverse primer as well as probe region were included in the analyses. The analysis showed that the Simplexa™ COVID-19 Direct target regions had no mismatch to 372,411 sequences (~98.6%) and were predicted to be detected by the assay based on sequence homology. There were 5213 (~1.4%) sequences with no mismatches for one gene oligo set (either ORF1ab or S gene), and there were 44 sequences (~0.01%) with mismatches in at least one primer or probe binding region, region in either ORF1ab or S gene target region. A Tm analysis was conducted as described above and the results demonstrated that detection of these sequences is not affected by the mismatches. Table 8 below summarizes the Tm analysis results.

An additional in silico inclusivity analysis was performed for complete SARS-CoV-2 genome sequences available in the GISAID database submitted from May 01, 2022 to July 31, 2022 including sequences of the Omicron BA.2.12.1, BA.2.75, BA.4 and BA.5 subvariants. The analysis included 211,224 sequences in the amplicon regions of the ORF1ab and S gene primer/probe regions. Only target sequences with full coverage of all three ORF1ab and S gene forward and reverse primer as well as probe region were included in the analyses. The analysis showed that the Simplexa™ COVID-19 Direct target regions had no mismatch to 208,582 sequences (~98.7%) and were predicted to be detected by the assay based on sequence homology. There were 2602 (~1.2%) sequences with no mismatches for one gene oligo set (either ORF1ab or S gene), and there were 40 sequences (~0.02%) with mismatches in at least one primer or probe binding region, region in either ORF1ab or S gene target region. A Tm analysis was conducted as described above and the results demonstrated that detection of these sequences is not affected by the mismatches. Table 8 below summarizes the Tm analysis results.

Table 8. Summary of Tm Analysis Results

Timeframe of Sequences Analyzed	Number of accessions in GISAID Database for the timeframe	Number of sequences where at least one target oligo set meets Tm criteria	Identity to SARS-CoV-2 gene design
Nov. 1, 2021 to Jan. 31, 2022	2,170,584	2,170,584	100%
Feb. 1, 2022 to Apr. 30, 2022	377,668	377,668	100%
May 1, 2022 to July 31, 2022	211,224	211,224	100%

Wet testing

Analytical reactivity was evaluated using five (5) strains of SARS-CoV-2 viral particles that were available. All five (5) strains of SARS-CoV-2 (Hong Kong/VM200001061/2020, England/204820464/2020, South Africa/KRISP-EC-K005325/2020, Japan/TY7-503/2021, hCoV19/USA/PHC658/2021) were detected at a concentration of 1000 copies/mL All replicates of the test samples had a result of "Detected" according to the interpretation algorithm.

Table 9. Analytical Reactivity Results

COVID-19 Strain	Tested Concentration	COVID-19 Qualitative Results % Detection (# Detected /#Tested)
Hong Kong/VM200001061/2020	1000 copies/mL	100% (3/3)
England/204820464/2020	1000 copies/mL	100% (3/3)
South Africa/KRISP-ECK005325/ 2020	1000 copies/mL	100% (3/3)
Japan/TY7-503/2021	1000 copies/mL	100% (3/3)
hCoV19/USA/PHC658/2021	1000 copies/mL	100% (3/3)

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. Analytical specificity/cross-reactivity was tested using 47 different viruses, bacteria, and fungi that could be found in nasopharyngeal swab specimens. In addition, pooled human nasal fluid was tested to represent diverse microbial flora. 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 typically 10^5 $TCID_{50}$/mL or PFU/mL or higher for viruses) into COVID-19 negative pooled NPS matrix collected in UTM All samples were tested in three replicates. The results from the cross-reactivity testing are summarized in Table 10.

Table 10: Laboratory Tested Cross-Reactivity Analysis

Organism	Tested Concentration[1]	Qualitative Results: % Detection (# Detected/#Tested)		
		S gene (FAM)	ORF1ab (JOE)	IC (Q670)
Adenovirus C (Type 1)	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Adenovirus 7A	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Bordetella pertussis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Candida albicans	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Chlamydophila pneumoniae	1 x 10^6 IFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Chlamydophila psittaci (genomic DNA)	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)

Organism	Tested Concentration[1]	Qualitative Results: % Detection (# Detected/#Tested)		
		S gene (FAM)	ORF1ab (JOE)	IC (Q670)
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Coxiella burnetii (genomic DNA)	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Cytomegalovirus	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Enterovirus 68	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Epstein-Barr virus	1 x 10^5 copies/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Escherichia coli	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Haemophilus influenzae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Human coronavirus 229E*	3 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Human coronavirus HKU1 (RNA)	1 x 10^5 genome copies/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Human coronavirus NL63*	3 x 10^4 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Human coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Human genomic DNA (Leukocytes)	1 x 10^6 cells/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Human metapneumovirus (hMPV)*	3 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Influenza A/Perth/16/2009	1 x 10^5 EID_{50}/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Influenza B/Florida/02/06	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Lactobacillus plantarum 17-5	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Legionella longbeachae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Legionella pneumophila	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Leptospira interrogans	1:10 Dilution	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)
Measles	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
MERS-coronavirus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Moraxella catarrhalis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Mumps	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Mycobacterium tuberculosis (genomic DNA)	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Mycoplasma pneumoniae	1 x 10^6 CCU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Neisseria elongata	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Neisseria meningitidis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Parainfluenza virus 1	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Parainfluenza virus 2	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Parainfluenza virus 3	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Parainfluenza virus 4	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Parechovirus 3	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Respiratory syncytial Virus A	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Respiratory syncytial Virus B	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Rhinovirus	1 x 10^5 U/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
SARS-coronavirus (RNA)	1 x 10^5 copies/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Staphylococcus aureus	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Staphylococcus epidermidis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Streptococcus pyogenes	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
Pooled Human Nasal Fluid	1:1 Dilution	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)

*A lower concentration was tested due to inability to obtain stock material with high titer
[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/milliliter

Bacillus anthracis, Influenza C and *Pneumocystis jirovecii* were not available. In addition, human coronavirus 229E, human coronavirus NL63 and human metapneumovirus (hMPV) were not available at a high enough concentration to permit wet testing at the concentration levels stated above. As a result of *in silico* BLAST analysis, the assay was found not to cross-react with these organisms.

POTENTIAL INTERFERING SUBSTANCES

Potential interfering substances from respiratory specimens were tested for ability to generate false negative results. The test samples contained inactivated SARS-CoV-2 at a concentration of 3x LoD in pooled negative nasopharyngeal swab matrix. Testing was performed with three (3) replicates per substance. The FluMist nasal vaccine was not tested as an interfering substance since it was unavailable at the time of the study.

Table 11: Potential Interfering Substances

Potentially Interfering Substance	Active Ingredient	Tested Concentration	COVID-19 Qualitative Results: % Detection (# Detected /#Tested)	IC Qualitative Results: % Detection (# Detected /#Tested)
Antibiotic nasal ointment (Mupirocin)	Mupirocin	6.6 mg/mL	100.0% (3/3)	100.0% (3/3)
Anti-viral drug (Oseltamivir)	Oseltamivir	3.3 mg/mL	100.0% (3/3)	100.0% (3/3)
Cold Eeze (Throat lozenges, Oral anesthetic and analgesic)	Zincum gluconicum 2X	2.5% (w/v)	100.0% (3/3)	100.0% (3/3)
Homeopathic allergy relief medicine	N/A	10% (v/v)	100.0% (3/3	100.0% (3/3)
Mucin (Bovine submaxillary gland, type I-S)	N/A	5 mg/mL	100.0% (3/3)	100.0% (3/3)
Nasal corticosteroids (Fluticasone)	Fluticasone	5% (v/v)	100.0% (3/3)	100.0% (3/3)
Allergy Relief Swabs (Nasal Gel, Zicam)	*Luffa opperculata, Galphimia glauca*, histaminum hydrochloricum, Sulphur	5% (w/v)	100.0% (3/3)	100.0% (3/3)
Nasal spray or drops (Oxymetazoline)	Oxymetazoline	15% (v/v)	100.0% (3/3)	100.0% (3/3)
Saliva	N/A	10% (v/v)	83.3% (5/6)	83.3% (5/6)
	N/A	5% (v/v)	100.0% (6/6)	100.0% (6/6)
Systemic antibacterial (Tobramycin)	Tobramycin	4 µg/mL	100.0% (3/3)	100.0% (3/3)
Whole Blood	N/A	2% (v/v)	100.0% (3/3)	100.0% (3/3)
Zanamivir	N/A	3 mg/mL	100.0% (6/6)	83.3% (5/6)

*Interference from saliva was observed at a concentration above 5%

mg = milligram, mL = milliliter, v/v = volume to volume, w/v = weight to volume, µg = microgram, NA = Not Applicable

INTERFERENCE BY OTHER MICROORGANISMS

The Simplexa™ COVID-19 Direct assay was evaluated by testing the ability to identify SARS-CoV-2 when other potentially inhibitory organisms were present. The panel of forty-seven (47) potentially inhibitory organisms was individually spiked into a pool with a low concentration at two times (2x) LoD of inactivated COVID-19 viral particles in NPS matrix. In addition, pooled human nasal fluid was tested to represent diverse microbial flora. For organisms not titered in CFU/mL or $TCID_{50}$/mL, other industry acceptable units were used as indicated. Samples were assayed in triplicate to screen for potential inhibition. No inhibition by other organisms was observed at the concentrations indicated in Table 12. Lactobacillus plantarum 17-5 showed interference when tested above (5×10^5 CFU/mL).

Table 12. Simplexa™ COVID-19 Direct – Microbial Interference

Organism	Tested Concentration[1]	COVID-19 Qualitative Results: % Detection (# Detected /#Tested)
Adenovirus C (Type 1)	1 x 10^5 U/mL	100.0% (3/3)
Adenovirus 7A	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
Bordetella pertussis	1 x 10^6 CFU/mL	100.0% (3/3)
Candida albicans	1 x 10^6 CFU/mL	100.0% (3/3)
Chlamydia pneumoniae	1 x 10^6 IFU/mL	100.0% (3/3)
Chlamydophila psittaci (genomic DNA)	2.5 x 10^5 copies/mL*	100.0% (3/3)
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	100.0% (3/3)
Coxiella burnetii (genomic DNA)	1 x 10^6 copies/mL	100.0% (3/3)
Cytomegalovirus	1 x 10^5 U/mL	100.0% (3/3)
Enterovirus 68	1 x 10^5 U/mL	100.0% (3/3)
Epstein-Barr virus	1 x 10^5 copies/mL	100.0% (3/3)
Escherichia coli	1 x 10^6 CFU/mL	100.0% (3/3)
Haemophilus influenzae	1 x 10^6 CFU/mL	100.0% (3/3)
Human coronavirus 229E	1.5 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
Human coronavirus HKU1 (RNA)	1 x 10^5 genome copies/mL	100.0% (3/3)
Human coronavirus NL63	1.5 x 10^4 U/mL*	100.0% (3/3)
Human coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
Human genomic DNA (Leukocytes)	1 x 10^6 cells/mL	100.0% (3/3)
Human metapneumovirus (hMPV)	1.5 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
Influenza A/Perth/16/2009	1 x 10^5 EID_{50}/mL	100.0% (3/3)
Influenza B/Phuket/3073/2013	1 x 10^5 $CEID_{50}$/mL	100.0% (3/3)
Lactobacillus plantarum 17-5**	5 x 10^5 CFU/mL	100.0% (6/6)
Legionella longbeachae	1 x 10^6 CFU/mL	100.0% (3/3)
Legionella pneumophila	1 x 10^6 CFU/mL	100.0% (3/3)
Leptospira interrogans	1:10 Dilution	100.0% (6/6)
Measles	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
MERS-coronavirus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
Moraxella catarrhalis	1 x 10^6 CFU/mL	100.0% (3/3)
Mumps	1 x 10^5 U/mL	95.0% (19/20)
Mycobacterium tuberculosis (genomic DNA)	1 x 10^6 copies/mL	100.0% (3/3)
Mycoplasma pneumoniae	1 x 10^6 CCU/mL	100.0% (3/3)
Neisseria elongata	1 x 10^6 CFU/mL	100.0% (3/3)
Neisseria meningitidis	1 x 10^6 CFU/mL	100.0% (3/3)
Parainfluenza virus 1	1 x 10^5 U/mL	100.0% (3/3)
Parainfluenza virus 2	1 x 10^5 U/mL	100.0% (3/3)
Parainfluenza virus 3	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
Parainfluenza virus 4	1 x 10^5 U/mL	100.0% (3/3)
Parechovirus 3	1 x 10^5 U/mL	100.0% (3/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	100.0% (3/3)
Respiratory syncytial virus A	5 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
Respiratory syncytial virus B	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
Rhinovirus	1 x 10^5 U/mL	100.0% (3/3)
SARS-Coronavirus (RNA)	1 x 10^5 copies/mL	100.0% (3/3)
Staphylococcus aureus	1 x 10^6 CFU/mL	100.0% (3/3)
Staphylococcus epidermidis	1 x 10^6 CFU/mL	100.0% (3/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	100.0% (3/3)
Streptococcus pyogenes	1 x 10^6 CFU/mL	100.0% (3/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	100.0% (3/3)
Pooled human nasal fluid	1:1 Dilution	100.0% (3/3)

*A lower concentration was tested due to inability to obtain stock material with high titer
**Interfernce with Lactobacillus plantarum 17-5 was observed at a concentration above 5 x 105 CFU/mL.
[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 per milliliter

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.

Conclusion

The analytical and method comparison studies have demonstrated that the Simplexa™ COVID-19 Direct is Substantially Equivalent to the predicate device (K211079). The device labeling is compliant with 21 CFR § 809.10.