EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SOLARIS Multiplex SARS-COV-2 ASSAY
(SOLARIS DIAGNOSTICS)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

The Solaris Multiplex SARS-CoV-2 Assay will be performed at Solaris Diagnostics, located at 110 Dewey Drive Suite A, Nicholasville KY 40356, which is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests, as described in the laboratory procedure that was reviewed by the FDA under this EUA.

INTENDED USE

The Solaris Multiplex SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens (nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates, bronchoalveolar lavage specimens) collected from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Solaris Diagnostics, located at 110 Dewey Drive Ste A, Nicholasville, KY 40356, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory 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 Solaris Multiplex SARS-CoV-2 Assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Solaris Multiplex SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Solaris Multiplex SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (real-time RT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates and bronchoalveolar lavage specimens from patients suspected of COVID-19 infection as recommended for testing by public health authority guidelines.

RNA is extracted from respiratory specimens using the MagMAX Viral/Pathogen Ultra kit (ThermoFisher Scientific, catalog number A42356) and the automated Kingfisher Flex DNA Extraction System, software version V.1.2.2. The MagMAX Viral/Pathogen Ultra kit uses bead-based technology for nucleic extraction.

For real-time RT-PCR, reagents include two primer and probe sets targeting the SARS-CoV-2 nucleocapsid (N) gene (N1 and N2) and a third primer and probe set targeting the RNase P housekeeping gene that is present in human cells. The primer/probe sets are used to prepare two PCR 'multiplex' reagent mixes containing the N1/RNase P primer/probe sets or the N2/RNase P primer/probe sets. Each specimen is evaluated using two wells, each with a different mix of primer/probe reagents. Extracted RNA is reverse transcribed and amplified by real-time PCR and the resulting amplicon is detected using the Applied Biosystems QuantStudio 5 with software version v1.5.1. In the amplification run, the forward and reverse primer binds to specific sequences with probe binding in the center. The N1 and N2 gene probe includes FAM dye along with quencher MGB and RNase P gene probe includes VIC dye along with quencher MGB. During the amplification process, the TAQ enzyme cleaves the probes causing the dye to separate from the quencher, generating a fluorescent signal.

INSTRUMENTS USED WITH TEST

The Solaris Multiplex SARS-CoV-2 Assay is to be used with the following Instruments:

- Tecan Evo 100 Liquid Handler, Software Evoware 2.8 (Specimen pipetting)
- Kingfisher Flex DNA Extraction System, software V.1.2.2 (Extraction)
- QuantStudio 5 Real time PCR System, Design and Analysis Software v1.5.1 (real-time RT-PCR)

REAGENTS AND MATERIALS

The following reagents are used with the real-time PCR portion of the Solaris Multiplex SARS-CoV-2 Assay (Table 1)

Table 1: Reagents Used for Real-Time RT-PCR

Material ID	Vendor	Catalog #/Storage
Custom Primer and Probe Sets (three primer/probe sets: N1, N2, and RNase P)	Life Technologies	Custom/ Store at -20°C
Luna universal probe one-step RT PCR Master Mix	New England BioLab	E3007E/ Store at -20°C
2019-nCoV_N-Positive Control	IDT	10006625/Store at -20°C

Additional Reagents/Components required to run the Solaris SARS-CoV-2 Assay:

- MagMAX Viral/Pathogen Ultra Kit Thermofisher Scientific Catalog# A42356 [Individual catalog numbers a) Elution Buffer (A42364), b) Pathogen Enzyme Mix (A42366), c) DNA/RNA Binding Beads (A42362), d) Proteinase K (A42363), e) Viral Pathogen Binding Solution (A42359), f) Viral Pathogen Wash Solution (A42360)]. All components are stored at RT.
- Nuclease-free water
- Plate Centrifuge
- 384-well optical reaction plate
- Optical Adhesive Sheet
- Vortexer
- Electronic pipettes
- PCR Workstation [UV lamp; Laminar flow (Class 100 HEPA filtered)]
- 200 Proof Ethanol
- DNase Free Water
- Tecan 1000 µL Tips
- Tecan Trough

CONTROLS TO BE USED WITH THE SOLARIS SARS-COV-2 ASSAY

The following assay controls are required for use with the Solaris Multiplex SARS-CoV-2 Assay:

1) Negative Control: The no template (Nuclease free water) control is performed with each batch to eliminate the possibility of contamination during the PCR run. This control is subjected to the entire testing process, including extraction.

2) Positive control: The positive control used with the assay is 2019-nCoV_N-Positive Control (IDT) which is comprised of quantified synthetic plasmid material. The control is diluted to 3X LOD and is tested in two appropriate wells

of the PCR amplification plate. The positive control is performed with each PCR test run to verify that the real-time PCR is performing as specified.

3) Negative Extraction control: The negative extraction control is a previously confirmed negative specimen that is included with each extraction batch to eliminate the possibility of sample contamination and to ensure successful RNA extraction.

4) Internal control: The human gene RNase P is used as an internal control for each clinical specimen to ensure the successful extraction of each specimen as well as to assess for proper assay setup, sample integrity, collection of human biological material, and assessment of potential interference.

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 2 for a summary of control results).

1) Quality Control Expected Results

- Negative Control: Negative for all targets (i.e., no amplification curves)
- Positive Control: Positive for N1, and N2 targets (Ct ≤37). Negative for RP Target (no amplification curve).
- Negative Extraction Control: Positive for RP target (Ct ≤37). Negative (no amplification curve) for N1, and N2 targets.

Table 2: Quality Control Results and Interpretation

Control Type	Used to Monitor	Expected Results			Expected Ct Values
		N1	N2	RNase P (RP)	
Negative	contamination during extraction and RT-PCR process	-	-	-	Undetected
Positive	amplification/primer-probe integrity	+	+	-	Ct ≤ 37 (N1, N2,) Undetected (RP)
Negative Extraction	cross-contamination during extraction, extraction/amplification for RP target	-	-	+	Undetected (N1, N2) Ct ≤ 37 (RP)

Internal Control (clinical specimens): Each clinical specimen that is negative for N1 and N2 targets should be positive for RP with a Ct value of ≤37. Repeat testing is indicated for any specimen that is negative for N1, N2, and RNase P targets.

If any of the controls fail, the test run is considered Invalid and patient specimen results cannot be interpreted. All specimens should be retested including the extraction step.

2) Examination and Interpretation of Patient Specimen Results:

Results from specimen testing are analyzed only after it is determined that all controls perform within the criteria described above. For clinical specimens, the assay cutoff for positivity is ≤37 Ct for each target. Table 3 lists all possible outcomes and actions needed.

Table 3: Solaris SARS-CoV-2 Assay Result Interpretation

SARS-CoV_N1	SARS-CoV_N2	RP	Results Interpretation	Report	Action
+	+	+ or -	SARS-CoV-2 detected	Positive	Reported to physician and public health authorities
If only one target is positive		+ or -	Inconclusive	Inconclusive	Sample repeated. If still Inconclusive, recollection recommended
-	-	+	SARS-CoV-2 detected	Negative	Reported to physician and public health authorities
-	-	-	Invalid	Invalid	Sample repeated. If still invalid, recollection recommended

Detected (+) = Ct ≤37, Not detected (-) = Ct >37

PERFORMANCE EVALUATION

1) Analytical Sensitivity (Limit of Detection):

Preliminary range-finding and confirmatory LoD testing were conducted with samples spiked with quantified genomic RNA from the SARS-CoV-2 isolate USA-WA1/2020 (NR-52285 BEI Resources). Samples were prepared using negative pooled clinical nasopharyngeal swab (NP) matrix. All testing was performed using the Tecan Evo for pipetting of specimens, followed by automated specimen extraction on the KingFisher instrument using the MagMAX Viral/Pathogen Ultra Kit (ThermoFisher Scientific, Cat# A42356). Specimen extracts were then tested by real-time RT-PCR on the Applied Biosystems QuantStudio 5, v1.5.1 software.

Preliminary range-finding testing included 10-fold dilutions with three replicates tested at each viral concentration. Results are presented in Table 4.

Table 4: LoD Study, Range-finding Results

Concentration copies/µL	Detection Rate		Mean Ct values	
	N1	N2	N1	N2
1 Copies/µL	0/3	0/3	N/A	N/A
10 Copies/µL	3/3	3/3	32.29	32.67
100 Copies/µL	3/3	3/3	29.19	29.37

Confirmatory LoD testing was conducted using 20 sample replicates prepared at both 10 copies/µL and 5 copies/µL. The LoD was confirmed as 10 copies/µl for the N1 target and 5 copies/µl for the N2 target. The LoD of the assay was confirmed as 10 Copies/µL (10,000 copies/mL); results are summarized in Table 5:

Table 5: LoD Study, Confirmatory Results

	2019-nCoV_N1		2019-nCoV_N2	
Copies/µL	**10**	**5**	**10**	**5**
Detected/Total	20/20	16/20	20/20	20/20
Ct (mean)	33.00	33.83	32.50	33.42
SD	0.66	13.91	0.83	1.41

2) Inclusivity/Exclusivity, In silico Analysis:

The Solaris Multiplex SARS-CoV-2 Assay primer and probe sequences are identical to the sequences for the FDA-authorized assay from CDC, 2019-Novel Coronavirus (2019-CoV) Real Time Diagnostic panel. Refer to EUA200001 for *in silico* analysis performed to evaluate inclusivity and exclusivity of the N1 and N2 target sequences.

3) Clinical Evaluation:

A clinical evaluation study of the Solaris Multiplex SARS-CoV-2 Assay was conducted with leftover clinical NP swab specimens that were previously collected for SARS-CoV-2 testing. All specimens were evaluated with an FDA-authorized comparator assay, the TaqPath COVID-19 Combo kit from ThermoFisher, catalog #A47814. Specimens were selected randomly. For the Solaris Multiplex SARS-CoV-2 Assay, RNA was extracted using the Tecan Evo instrument for specimen pipetting, the KingFisher instrument and MagMAX Viral/Pathogen Ultra Kit (ThermoFisher Scientific, Cat# A42356) for extraction, and the Applied Biosystems QuantStudio5 for real-time RT-PCR. Results from the study demonstrated 100% PPA and NPA when compared to results from the comparator assay as shown in Table 6.

Table 6: Clinical Evaluation Study, Performance Summary

Solaris Multiplex SARS-CoV-2 Assay	**FDA EUA Assay**	
	Positive	Negative
Positive	30	0
Negative	0	30

Positive Percentage Agreement: 30/30 (100%)
Negative Percentage Agreement: 30/30 (100%)

An additional clinical evaluation study was conducted with contrived specimens. Specimens were prepared using individual NP Swab specimens that were determined to be negative for SARS-CoV-2. For contrived positive specimens, each NP swab specimens was spiked with whole viral SARS-CoV-2 isolate USA-WA1/2020 (NR-52285 BEI Resources. For contrived negative specimens, 30 individual negative NP swab specimens were tested. The 30 spiked positive specimens included 10 specimens at 1X LOD, 10 specimens at 2X LOD, 5 specimens at 5X LOD, and 5 specimens at 10X LOD. All samples were tested on Solaris Multiplex SARS-CoV-2 Assay after the extraction step using the MagMAX Viral/Pathogen Ultra Kit, ThermoFisher Scientific, Catalog: A42356 on the KingFisher Instrument and that real-time RT-PCR was performed using the Applied Biosystems QuantStudio 5 with software version v1.5.1. The testing of contrived clinical specimens demonstrated 100% agreement for both spiked and negative samples. The data from the study is presented in Table 7.

Table 7: Clinical Evaluation, Contrived Specimens

RNA Conc (Copies/ µl)	**Number of NP Swabs**	**NCOV-N1 % Positive (Mean Ct values)**	**NCOV-N2 % Positive (Mean Ct values)**	**RP (IC) % Positive (Mean Ct values)**
1X LOD (10)	10	100 % (33.08)	100 % (33.95)	100 % (21.87)
3X LOD (20)	10	100 % (28.82)	100 % (29.84)	100 % (27.47)
10X LOD (50)	5	100 % (20.23)	100 % (20.52)	100 % (25.23)
50X LOD (100)	5	100 % (16.97)	100 % (17.51)	100 % (29.02)
Non-Spiked	30	0% (40)	0% (40)	100 % (26.21)

Positive Percentage Agreement: 30/30 (100%)
Negative Percentage Agreement: 30/30 (100%)

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Solaris Diagnostics located at 110 Dewey Drive Ste A, Nicholasville, KY 40356;

- This test has been authorized only for the detection of nucleic acid from SARS CoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method used was MagMAX Viral/Pathogen Ultra kit on the automated Kingfisher Flex DNA Extraction System, software version V.1.2.2 and the assay was Solaris Multiplex SARS-CoV-2 Assay on the Applied Biosystems QuantStudio 5 with software version v1.5.1. The results are summarized in the following Table.

Table 8. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not Detected