ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
The SDI SARS-CoV-2 Assay
(SDI Laboratories)

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

(The SDI SARS-CoV-2 assay will be performed at the Specialty Diagnostic (SDI Laboratories) in Garden Grove, CA, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The SDI SARS-CoV-2 assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and oropharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing using this assay is limited to SDI Laboratories, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

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. 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 SDI SARS-CoV-2 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. The SDI SARS-CoV-2 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SDI SARS-CoV-2 assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The primer and probe set, used in this test is identical to that described in the BGI emergency use authorized assay (EUA200034) and detects RNA from the SARS-CoV-2 in nasopharyngeal or oropharyngeal swabs from individuals suspected of COVID-19 by their healthcare provider.

The test uses one primer and probe set to detect one region in the SARS-CoV-2 ORF1a/b gene and one primer and probe set to detect human β-actin in a clinical sample. RNA isolated from nasopharyngeal or oropharyngeal swabs is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems 7500 (ABI7500) instrument with software version 2.0.5. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (BHQ1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by ABI7500.

INSTRUMENTS USED WITH TEST

The SDI SARS-CoV-2 assay is to be used with AusDx MT-Prep Extraction System and Applied Biosystems 7500 instrument with software version 2.0.5.

REAGENTS AND MATERIALS

Reagent	**Manufacturer**	**Catalog #**
2019-nCoV Reaction Mix	BGI	MFG030010
2019-nCoV Enzyme Mix	BGI	MFG030010
2019-nCoV Positive Control	BGI	MFG030010
2019-nCoV Blank Control	BGI	MFG030010

Instrument	**Manufacturer**	**Catalog #**
AusDx MT-Prep Extraction System	AUSdiagnostics	194408
Applied Biosystems 7500	Applied Biosystems	4345241
ABI MicroAmp Optical 96-Well Fast Clear Reaction Plate	Applied Biosystems	4483485
AusDx extraction cartridges	AUSdiagnostics	93011

CONTROLS TO BE USED WITH THE COV-19 IDx Assay

1) A no template control (NTC) is needed to monitor the possibility of sample contamination on the assay run. This is processed beginning at the extraction step where 200 µL are added to the AusDx extraction cartridge. The NTC is molecular grade, nuclease-free water.

2) A positive extraction control (PEC) is needed to verify that the assay run is performing as intended. The PEC is added directly to the AusDx extraction cartridge at a volume of 200 µL. The positive control is provided in the BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV (EUA200034) and is comprised of a mixture of non-infectious MS2-phage-based pseudo-virus containing the SARS-CoV-2 RNA target sequence (104 copies/mL) and human β-

actin RNA transcripts (105 copies/mL). The SARS-CoV-2 target sequence is packed in MS2-phage envelope protein as a surrogate for native virus.

3) An internal control (IC) targeting β-actin is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This serves as the extraction positive control to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

1) COV-19 IDx test Controls – Positive, Negative, and Internal:

PEC – positive for SARS-CoV-2 ORF1a/b target detected (Ct < 35), positive for β-actin target (Ct < 35)
IC – negative for SARS-CoV-2 ORF1a/b target (Ct ≥ 37), positive for β-actin target (Ct < 35)
NTC – negative for SARS-CoV-2 ORF1a/b (Ct ≥ 37), negative for β-actin target (Ct ≥ 35)

If any control does not perform as described above, run is considered invalid and all specimens are repeated from extraction step.

2) Examination and Interpretation of Patient Specimen Results:

IC – all clinical samples should yield positive results for β-actin target at < 35 Ct. Samples that fail to show detection of IC within this range and SARS-CoV-2 ORF1a/b target should be repeated from extraction step. If sample detects the SARS-CoV-2 ORF1a/b target, the lack of amplification of RP target can still be valid.

SDI SARS-CoV-2 Assay results interpretation

SARS-CoV-2 N1	B-actin	Result Interpretation	Report	Actions
+	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate public health authorities.
-	+	SARS-CoV-2 Not Detected	NEGATIVE	Report results to sender.
-	-	Invalid Result	INVALID	Repeat extraction and RT-PCR. If a second test yields "INVALID", report results to sender and request a second sample if clinically indicated.

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µL) that can be detected by the SDI SARS-CoV-2 assay test at least 95% of the time. The preliminary LoD was established by testing eight different dilutions of pseudovirus containing SARS-CoV-2 RNA[1] (50 cp/µL, 25 cp/µL, 12.5 cp/µL, 5 cp/µL, 2.5 cp/µL, 1.25 cp/µL, 0.5 cp/µL, and 0.25 cp/µL) in triplicate by one operator and then singlet by a second operator. The preliminary LoD was determined to be 0.5 cp/µL for NP/OP matrix[2] collected in both UTM and SDI Swab media[3]. The preliminary LoD was confirmed by testing 20 replicates of 0.5 cp/µL. The samples at 0.5 cp/µL were prepared by spiking pseudovirus into NP/OP clinical matrix, collected in UTM or SDI Swab media. The study results showed that the LoD of the SDI SARS-CoV-2 assay is 0.5 cp/µL (20/20 positive) for specimens collected in either UTM or SDI Swab media.

Inclusivity:

The primer/probe set for the ORF1a/b SARS-CoV-2 target was designed by BGI, which conducted the *in silico* inclusivity analysis on known sequences of SARS-CoV-2 and by wet testing of 10 specimens from different geographical regions of China that were confirmed as SARS-CoV-2 positive. The data from this analysis is available in the FDA EUA EUA200034 “THE BGI GENOMICS REAL-TIME FLUORESCENT RT-PCR KIT”

2) ***Analytical Specificity:***

The primer/probe set for the ORF1a/b SARS-CoV-2 target was designed by BGI which conducted the cross-reactivity testing *in silico* and by wet-testing. The data from this analysis is available in the FDA EUA EUA200034 “THE BGI GENOMICS REAL-TIME FLUORESCENT RT-PCR KIT”.

Cross-reactivity of the SDI SARS-CoV-2 assay was also evaluated by wet-testing, in house. A volume of 200 µL of each pathogen (Table 1) was extracted, as provided from Zeptometrix, using the AusDx MT-Prep Extraction System and tested for cross-reactivity on the ABI 7500. The cross-reactivity results for SDI SARS-CoV-2 Assay are summarized in Table 4.

[1] The pseudovirus used to perform the analytical and clinical evaluation of the SDI SARS-CoV-2 Assay is the same material provided by BGI as the positive control (see EUA200034 for more information).
[2] Nasopharyngeal (NP) and oropharyngeal (OP) specimens were collected and were not differentiated for the purpose of clinical and analytical testing.
[3] ThinPrep liquid cytology fixative

In addition to individual testing, pathogens were combined into groups of 3 or 4 and tested for SARS-CoV-2 using the SDI SARS-CoV-2 assay (Table 2). The pathogen panels (Group 1-6) were also spiked to 5 cp/µL with pseudovirus and tested using the SDI SARS-CoV-2 assay to show there is no interference from similar respiratory pathogens (Table 2).

Table 1. Pathogens[a] tested for cross reactivity against the ORF1a/b Primers/Probe set.

Designation	Organism	ORF1a/b Target	Strain
MH1	Influenza A HIN1	Not Detected	A/New Caledonia/20/99
MH2	Influenza A H3	Not Detected	A/Brisbane/10/07
MH3	Influenza A 2009 HlNl pdm	Not Detected	A/NY/02/09
MH4	Influenza B	Not Detected	B/Florida/02/06
MH5	Metapneumovirus 8	Not Detected	Peru6-2003
MH6	Respiratory Syncytial virus A	Not Detected	
MH7	Rhinovirus 1A	Not Detected	
MH8	Parainfluenza virus Type 1	Not Detected	
MH9	Parainfluenza virus Type 2	Not Detected	
MH10	Parainfluenza virus Type 3	Not Detected	
MH11	Parainfluenza virus Type 4	Not Detected	
MH12	Adenovirus Type 3	Not Detected	
MH13	Coronavirus NL63	Not Detected	
MH14	Coronavirus 229E	Not Detected	
MH15	Coronavirus 0C43	Not Detected	
MH16	Coronavirus HKU-1	Not Detected	
MH17	*M. pneumnoniae*	Not Detected	M129
MH18	*C. pneumoniae*	Not Detected	CWL-029
MH19	*B. pertussis*	Not Detected	A639
MH20	Adenovirus Type 31	Not Detected	
MH21	Adenovirus Type 1	Not Detected	
MH22	*B. parapertussis*	Not Detected	A747
MH23	Negative matrix	Not Detected	

[a]Zeptometrix NATrol Respiratory verification Panel 2 (Cat# NATRVP2-BIO).

Table 2. Multi-panel pathogens tested for cross-reactivity and microbial interference against the ORF1a/b Primers/Probe set.

Group	Samples	Spiked (cp/uL)	Results
1	MH1, MH10, MH11, MH12	0	Not Detected
2	MH12, MH14, MH15, MH16	0	Not Detected
3	MH17, MH18, MH19, MH2,	0	Not Detected

	MH20		
4	MH21, MH22, MH23	0	Not Detected
5	MH3, MH4, MH5,MH6	0	Not Detected
6	MH7, MH8, MH9	0	Not Detected
1	MH1, MH10, MH11, MH12	5	Detected
2	MH12, MH14, MH15, MH16	5	Detected
3	MH17, MH18, MH19, MH2, MH20	5	Detected
4	MH21, MH22, MH23	5	Detected
5	MH3, MH4, MH5, MH6	5	Detected
6	MH7, MH8, MH9	5	Detected

3) Clinical Evaluation:

A contrived clinical study was conducted to evaluate the performance of the SDI SARS-CoV-2 assay. The 30 positive samples were prepared by spiking pseudovirus into individual NP/OP matrix, collected in either UTM and SDI Swab media at 1X, 10X, and 1,000X LoD. Samples were extracted and tested in a randomized and blinded fashion using the AusDx automated purification system and ABI 7500 instrument. The positive and negative percent agreements between the SDI SARS-CoV-2 assay and the expected results are shown below for each collection media type.

Table 3. Clinical performance of the SDI SARS-CoV-2 assay on the ABI 7500 for specimens collected in UTM:

SARS-CoV-2 concentration	Number of NP/OP swabs	ORF1a/b target % Positive (95% CIs)
1x LoD	10	10/10 100% (72.3 – 100)
10x LoD	10	10/10 100% (72.3 – 100)
1000x LoD	10	10/10 100% (72.3 – 100)
Negative	30	0/30 (N/A)

NA = Not available

Performance of the SDI SARS-CoV-2 assay on the ABI 7500 for samples collected in UTM against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)

Table 4. Clinical performance of the SDI SARS-CoV-2 assay on the ABI 7500 for specimens collected in SDI Swab media:

SARS-CoV-2 concentration	Number of NP/OP swabs	ORF1a/b target % Positive (95% CIs)
1x LoD	10	10/10 100% (72.3 – 100)
10x LoD	10	10/10 100% (72.3 – 100)
1000x LoD	10	10/10 100% (72.3 – 100)
Negative	30	0/30 (N/A)

N/A = Not available.

Performance of the SDI SARS-CoV-2 assay on ABI 7500 for samples collected in SDI Swab media against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)

Additionally, five positive and five negative patient samples were sent to XymBio and tested using the TaqPath COVID-19 Combo Kit (ThermoFisher). All results were concordant.

Table 5. Result Verification

Sample	SDI Test Date	Result	XymBio Test Date	Result
1	4/5/2020	Positive	4/6/2020	Positive
2	4/5/2020	Positive	4/6/2020	Positive
3	4/5/2020	Positive	4/6/2020	Positive
4	4/5/2020	Positive	4/6/2020	Positive
5	4/5/2020	Positive	4/6/2020	Positive
6	4/5/2020	Negative	4/6/2020	Negative
7	4/5/2020	Negative	4/6/2020	Negative
8	4/5/2020	Negative	4/6/2020	Negative
9	4/5/2020	Negative	4/6/2020	Negative
10	4/5/2020	Negative	4/6/2020	Negative