EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
The COV-19 IDx assay
(Ipsum Diagnostics, LLC)

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

(The COV-19 IDx assay will be performed at the Ipsum Diagnostics, LLC in Atlanta, Georgia, 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 COV-19 IDx 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 is limited to Ipsum Diagnostics, LLC, or other laboratories designated by Ipsum Diagnostics, LLC that are also 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 COV-19 IDx 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 COV-19 IDx assay is only for use under the Food and Drug Administration’s Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COV-19 IDx assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test uses one primer and probe set to detect one region in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. RNA isolated from nasopharyngeal or oropharyngeal swabs is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems QuantStudio12 Flex (QS12) instrument with software version 1.2.2. 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 QS12.

INSTRUMENTS USED WITH TEST

The COV-19 IDx assay is to be used with the KingFisher Flex nucleic acid extraction systems using the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit and QS12 instrument with software version 1.2.2.

REAGENTS AND MATERIALS

Reagent	**Manufacturer**	**Catalog #**
the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit	ThermoFisher	M6246-03
PrimeDirectTM Probe RT-qPCR Mix	TaKarRa	RR650A
COVID-19_N1-F Primer	IDT	Custom
COVID-19_N1-R Primer	IDT	Custom
COVID-19_N1-P Probe	IDT	Custom
RP-F Primer	IDT	Custom
RP-R Primer	IDT	Custom
RP-P Probe	IDT	Custom
COVID-19_N_Positive Control	IDT	10006625

CONTROLS TO BE USED WITH THE COV-19 IDx Assay

a) A no template control (NTC) is needed to monitor the possibility of sample contamination on the assay run and is used on every assay plate. This control is molecular grade, nuclease-free water.

b) A positive extraction control (PEC) is needed to verify that the assay run is performing as intended and is added to pooled negative patient sample collected in UTM, presumed negative for SARS-CoV-2, before the extraction at a concentration of 50 cp/µL. The positive control is the 2019-nCoV_N_Positive Control (Integrated DNA Technologies, CAT#: 10006625) containing a DNA sequence of the nCoV nucleocapsid gene which is the target of the N1 CDC-designed primers/probe.

c) An internal control (RP) targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. Because control *(b)* is a DNA template, this serves as a control to ensure that the reverse transcription step is proceeding as intended. This also serves as the extraction positive control to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

d) A negative extraction control (NEC) consists pooled negative patient sample collected in UTM, presumed negative for SARS-CoV-2. It serves both as a negative extraction control to monitor for any cross-contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction.

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:

NTC – negative for all targets detected (Ct ≥35)
PEC – positive for SARS-CoV-2 N1 target detected (Ct < 35)
RP – negative for SARS-CoV-2 N1 target (Ct ≥35), positive for RNase P (RP) target (Ct < 35)
NEC – negative for SARS-CoV-2 targets (Ct ≥35), positive for RNase P (RP) 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:

RP – all clinical samples should yield positive results for RP target at < 35 Ct. Samples that fail to show detection of RP and the N1 SARS-CoV-2 target within this range should be repeated from extraction step. If the assay detects the N1 SARS-CoV-2 target, the lack of amplification of RP target can still be valid.

COV-19 IDx test results interpretation

SARS-CoV-2 N1	RNase P	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 additional clinical sample is unavailable, report Invalid Results, which will request a new specimen be collected. If a second test yields "INVALID", report results to sender.

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 COV-19 IDx test at least 95% of the time. The preliminary LoD was established by testing serial dilutions of genomic RNA from BEI ATCC Genomic RNA from SARS Related Coronavirus 2 (Catalog No. NR-52285) into nasopharyngeal (NP) clinical matrix, presumed negative, in quadruplicate (3415 cp/µL, 341 cp/µL, 34 cp/µL, 17 cp/µL, 8.5 cp/µL, 4.3 cp/µL). The preliminary LoD was determined to be 8.5 cp/µL (4/4 positive). The preliminary LoD was confirmed by testing 20 replicates of 8.5 cp/µL. The samples at 8.5 cp/µL were prepared by spiking genomic RNA into nasopharyngeal (NP) clinical matrix, presumed negative. The study results showed that the LoD of the COV-19 IDx assay is 8.5 cp/µL (20/20 positive).

Inclusivity:

The primer/probe set for the N1 SARS-CoV-2 marker was designed by the CDC which conducted the *in silico.* Inclusivity analysis on known sequences of SARS-CoV-2. The data from this analysis is available in the FDA EUA EUA200001 "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel"

2) ***Analytical Specificity:***

The primer/probe set for the N1 SARS-CoV-2 marker was designed by the CDC which conducted the cross-reactivity testing *in silico.* The data from this analysis is available in the FDA EUA EUA200001 "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel"

Cross-reactivity of the COV-19 IDx test was also evaluated using purified nucleic acid from a panel of organisms listed in table 4. There was no cross-reactivity observed for any of the tested pathogens. Cross-reactivity is defined as Ct < 35.

Cross-reactivity test results:

Organism	N1 Target	Source	Strain	Concentration
Adenovirus 3	Not Detected	Zeptometrix	N/A	NC
B. pertussis	Not Detected	Zeptometrix	A639	NC
C. pneumoniae	Not	Zeptometrix	CWL-029	NC

	Detected			
Coronavirus 229E	Not Detected	Zeptometrix	N/A	NC
Coronavirus NL63	Not Detected	Zeptometrix	N/A	NC
Coronavirus OC43	Not Detected	Zeptometrix	N/A	NC
Influenza A H1N1pdm	Not Detected	Zeptometrix	A/NY/02/09	NC
Influenza A H1N1	Not Detected	Zeptometrix	A/New Caledonia/20/99	NC
Influenza H3N2	Not Detected	Zeptometrix	A/Brisbane/10/07	NC
Influenza B	Not Detected	Zeptometrix	B/Panama/45/90	NC
M. pneumoniae	Not Detected	Zeptometrix	M129	NC
Metapneumovirus 8	Not Detected	Zeptometrix	Peru6-2003	NC
Parainfluenza 1	Not Detected	Zeptometrix	N/A	NC
Parainfluenza 2	Not Detected	Zeptometrix	N/A	NC
Parainfluenza 3	Not Detected	Zeptometrix	N/A	NC
Parainfluenza 4	Not Detected	Zeptometrix	N/A	NC
Rhinovirus 1A	Not Detected	Zeptometrix	N/A	NC
RSV A	Not Detected	Zeptometrix	N/A	NC
SARS-coronavirus	Not Detected	IDT	N/A	2×10^5 cp/µL
MERS-coronavirus	Not Detected	IDT	N/A	2×10^5 cp/µL
Streptococcus pneumoniae	Not Detected	ThermoFisher	N/A	1×10^7 cp/µL
Streptococcus pyogenes	Not Detected	ThermoFisher	N/A	1×10^7 cp/µL
Candida albicans	Not Detected	ThermoFisher	N/A	1×10^7 cp/µL
Pseudomonas	Not	ThermoFisher	N/A	1×10^7 cp/µL

aeruginosa	Detected			
Staphylococcus epidermis	Not Detected	ThermoFisher	N/A	1 x 10^7 cp/μL
Coronavirus HKU-1	Not Detected	Zeptometrix	Recombinant	NC

N/A = Not Available
NC = No concentration. Zeptometrix NATtrol Respiratory Verification panel. All concentrations are listed on the SDS as proprietary.

3) Clinical Evaluation:

A contrived clinical study was conducted to evaluate the performance of the COV-19 IDx test. A total of 66 individual nasopharyngeal clinical specimens, collected in UTM and presumed negative for SARS-CoV-2, were used in this study. A total of 30 negative and 36 contrived positive samples were tested. Positive samples were prepared by spiking BEI ATCC Genomic RNA from SARS Related Coronavirus 2 (Catalog No. NR-52285) into NP matrix mixed with lysis buffer from the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit at 1X, 2X, and 4X LoD. Samples were extracted and tested in a randomized and blinded fashion using the KingFisher Flex nucleic acid extraction system and QS12 instrument. The positive and negative percent agreements between the COV-19 IDx assay and the expected results are shown below.

Clinical performance of the COV-19 IDx test with NP swabs:

SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)
1x LoD	12	12/12 100% (75.8 – 100)
2x LoD	12	12/12 100% (75.8 – 100)
5x LoD	12	12/12 100% (75.8 – 100)
Negative	30	0/30 (NA)

NA = Not available

Performance of the COV-19 IDx test on QuantStudio 12K Flex against the expected results are:

Positive Percent Agreement 36/36 = 100% (95% CI: 90.4% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)