ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY INFORM DIAGNOSTICS SARS-COV-2 RT-PCR ASSAY (INFORM DIAGNOSTICS, INC.)

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

(The Inform Diagnostics SARS-CoV-2 RT-PCR Assay will be performed at Inform Diagnostics, Inc. certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The Inform Diagnostics SARS-CoV-2 RT-PCR Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swabs, as well as nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Inform Diagnostics, Inc. located in Phoenix, AZ 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 detection and identification of SARS-CoV-2 RNA. The 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Inform Diagnostics SARS-CoV-2 RT-PCR Assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Inform Diagnostics SARS-CoV-2 RT-PCR Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Inform Diagnostics SARS-CoV-2 RT-PCR Assay is a real-time reverse transcription polymerase chain reaction test. The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel and are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients suspected of COVID-19 by their healthcare provider. The test uses two primer and probe sets to detect two regions in the SARS-CoV-2 nucleocapsid (N) gene (N1 and N2), and one primer and probe set to detect human RNase P (RP) in control samples and clinical specimens. Three separate master mixes for each target are prepared and run with the Inform Diagnostics Assay.

RNA is isolated from respiratory specimens including nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swabs as well as nasopharyngeal wash/aspirate or nasal aspirates and BAL specimens using the ViralXpress DNA/RNA Extraction Reagent (Millipore, Cat # 3095). Nucleic acid is manually extracted from 50 µL of acceptable specimen with the addition of carrier RNA into the lysis buffer. RNA is reverse transcribed to cDNA and subsequently amplified using either the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with software version 1.4 or the QuantStudio with software version 1.0.3. 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 (BHQ-1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The Inform Diagnostics SARS-CoV-2 Assay is to be used with the following Applied Biosystems PCR platforms:

- 7500 Fast Dx Real-Time PCR Instrument (ABI7500) with software version 1.4
- QuantStudio with software version 1.0.3

The QIAgility (Qiagen, Cat # Q080957) with software version 4.17.1 is used to prepare and dispense the RT-PCR master mix into the reaction plate.

REAGENTS AND MATERIALS

Reagent Manufacturer and Description	Catalog #	Manufacturer
ViralXpress DNA/RNA Extraction Reagent	3095	Millipore
Carrier RNA	1017647	Qiagen
TaqPath 1-Step RT-qPCR Mater Mix, CG	A15300, A15299	ThermoFisher Scientific
COVID-19_N1-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N1-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N1-P Probe (N1 probe)	10006606	Integrated DNA Technologies
COVID-19_N2-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N2-R Primer (reverse primer)	10006606	Integrated DNA Technologies

Reagent Manufacturer and Description	Catalog #	Manufacturer
COVID-19_N2-P Probe (N2 probe)	10006606	Integrated DNA Technologies
RP-F Primer (forward primer)	10006606	Integrated DNA Technologies
RP-R Primer (reverse primer)	10006606	Integrated DNA Technologies
RP-P Probe (RNase P probe)	10006606	Integrated DNA Technologies
Hs_RPP30 Positive Control	10006626	Integrated DNA Technologies
2019-nCoV_N_Positive Control	10006625	Integrated DNA Technologies
MicroAmp Optical 96-Well Reaction plate	4481192	Life Technologies
MicroAmp Optical Adhesive Film	4311971	Life Technologies

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

1) A no template control (NTC) is needed to check for contamination of RT-PCR assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used on every assay plate.

2) The positive control is prepared using the 2019-nCoV_N_Positive Control (IDT, Cat # 10006625) and Hs_RPP30 Positive Control (IDT, Cat # 10006626). Positive template control is needed to verify PCR reagent integrity as well as proper assay set-up of the RT-PCR reactions for the N1, N2, and RNase P genes. The positive control is used on every assay plate starting at master mix addition at a final N1, N2, and RNase P template concentration of 50 copies/µL. The 2019-nCoV_N_Positive Control is commercially supplied from IDT and is made of *in vitro* transcribed and purified plasmid DNA targets that contains one copy each of N1 and N2. The Hs_RPP30 Positive Control is RNase P template supplied from IDT that Inform Diagnostics also incorporates into their SARS-CoV-2 positive control.

3) A negative extraction (NEC) control is a nasopharyngeal swab sample from a previously confirmed SARS-CoV-2 negative patient. This control is used as a negative control as well as an extraction control to monitor for any cross-contamination during the analytical process and to verify the success of RNA extraction and sample integrity. A NEC is used in each extraction batch.

4) RNase P is co-extracted and amplified from all patient samples as an internal control to assess the extraction efficiency and specimen quality. This also serves as an extraction control to ensure that samples resulting as negative contain nucleic acid for testing. Detection of the RNase P gene in patient test samples verifies successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection. No additional RNase P is added to clinical samples prior to performing the extraction procedure.

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 1 for a summary of control results).

1) *COVID-19 RT-PCR Test Controls – Positive, Extraction, Internal, and NTC:*

- No template controls should be negative (Ct Not Detected) for all targets. If the N1, N2, or RNase P targets exhibit positive fluorescence above the threshold (Ct ≤ 40), it is possible that contamination occurred, or that the assay was setup improperly. The RT-PCR run is invalid. The user is instructed to repeat the RT-PCR using residual extracted material. If the repeat NTC result is positive for any of the assay targets, re-extract residual clinical specimens and fresh controls and re-test all samples.

- The positive control contains both the 2019-nCoV_N_Positive Control and Hs_RPP30 and should therefore, be positive for the N1 and N2 targets (Ct ≤ 40) as well as the RNase P target (Ct ≤ 40). Negative results with N1, N2, or RNase P targets invalidates the run and suggests the assay may have been set up incorrectly, the integrity of the primers/probes could have been compromised, or potential carry-over of PCR inhibitors. The user is instructed to repeat the RT-PCR step using residual extracted material. If the repeat test result is negative for the N1/N2 and/or RNase P targets, re-extract residual clinical specimens and fresh controls and repeat RT-PCR.

- The negative extraction control (negative clinical sample) should be negative for N1, N2 (Ct Not Detected or Ct > 40), and positive for the RNase P target (Ct ≤ 40). If positive results are obtained for N1 and N2 targets, contamination of nucleic acid extraction reagents or cross-contamination of samples may have occurred. Failure of the control to yield a RNase P Ct value of ≤ 40 may indicate improper extraction of nucleic acid, carry-over of PCR inhibitors, or insufficient cellular material. The extraction run and the RT-PCR run are invalid and should be repeated using new extracted material from residual patient samples and fresh controls.

- RNase P is co-extracted and amplified from all patient samples as an internal control to assess the extraction efficiency and specimen quality. This also serves as a positive extraction control to ensure that samples resulting as negative contain nucleic acid for testing. Detection of the RP gene in patient test samples verifies successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection.

Table 1. Ct Values for Controls that Must be Observed to Obtain Valid Results

Control	Expected N1 Result	Expected N2 Result	Expected RP Result
2019-nCoV_N_ Positive Control with Hs_RPP30 Positive Control (N1, N2, RP template)	Ct ≤ 40	Ct ≤ 40	Ct ≤ 40
No Template Control (NTC)	Not Detected	Not Detected	Not Detected
Negative Extraction Control	Not Detected	Not Detected	Ct ≤ 40
Internal RNase P Control	Not Detected	Not Detected	Ct ≤ 40 / Not Detected*

Not Detected; no detectable signal or Ct > 40

If the results obtained with the Positive, Internal, and No Template Controls do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed using residual extracted nucleic acid. If the negative extraction control does not meet the acceptability criteria, all specimens in the batch should be re-extracted from residual clinical samples and the RT-PCR assay should be re-run.

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 2) for guidance on interpretation and reporting of results.

Table 2: Interpretation of Patient Results Using the Inform Diagnostics SARS-CoV-2 RT-PCR Test

N1 (Ct ≤ 40)	N2 (Ct ≤ 40)	RNase P (Ct ≤ 40)	Interpretation	Report Result	Actions
+	+	+/-*	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities.
+	-	+/-*	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities.
-	+	+/-*	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities.
-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Reported to sender and appropriate public health authorities.
-	-	-	**Invalid test**	**INVALID**	Repeat extraction and RT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

*If either N1 or N2 is positive, it is not necessary to have positive signal from the RNase P probe.

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):

The LoD of the Inform Diagnostics Test was determined using synthetic SARS-CoV-2 viral RNA from Twist Bioscience (Cat # MT007544.1). A preliminary LoD was determined by testing serial dilutions (1000 copies/µL – 10 copies/µL) of synthetic

RNA spiked into pooled clinical negative, nasopharyngeal swab or oropharyngeal swab matrix using either one replicate or three replicates at each target level. Spiked samples were tested with the Inform Diagnostics Test following extraction with the ViralXpress Reagent on both claimed PCR instruments. Two preliminary concentrations of 20 copies/µL and 15 copies/µL were chosen for confirmatory testing with 20 individual extraction replicates on both the ABI7500 and QuantStudio platforms.

The established LoD of the Inform Diagnostics SARS-CoV-2 RT-PCR Assay was 20 copies/µL. The results of the LoD confirmatory study are summarized below.

Table 3. LoD Verification Study Results

Instrument	Concentration (copies/µL)	Average Ct Values		# Detected / Total Tested	
		N1	N2	N1	N2
ABI7500	20	30.21	36.22	20/20	20/20
	15	34.60	37.61	8/20	8/20
QuantStudio	20	33.50	37.14	20/20	20/20
	15	34.50	38.12	13/20	10/20

2) *Analytical Inclusivity/Specificity:*

Inclusivity:

The Inform Diagnostics SARS-CoV-2 RT-PCR Assay utilizes identical oligonucleotide sequences for the N1 and N2 target genes to those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The inclusivity and cross-reactivity of the CDC EUA assay has been evaluated previously and therefore, additional evaluation was not necessary. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (FDA submission number EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

Since the alignments of the CDC's primers/probes were completed in February 2020, an additional in silico inclusivity analysis was completed to assess the predicted inclusivity to other deposited SARS-CoV-2 sequences in the NCBI database. In silico testing was performed using BLASTN 2.10.1 on May 12, 2020 and confirmed 100% nucleotide identity of the N1 and N2 primers and probes to all available SARS-CoV-2 partial and complete genomes published by NCBI.

Exclusivity:

To assess for potential cross-reactivity of the Inform Diagnostics Test, an *in silico* analysis of the SARS-CoV-2 N1 and N2 primer and probe sequences was performed using BLASTN 2.10.1 against partial or complete genomes of other common respiratory viral and bacterial pathogens listed in Table 4. None of the pathogen sequences displayed greater than 80% homology with any of the SARS-CoV-2 N1 and N2 primers/probes.

Table 4. *In Silico* Cross-Reactivity Analysis of N1 and N2 Oligonucleotides

Pathogen Name	GenBank Accession ID	Genome	N1 Homology	N2 Homology
Human coronavirus 229E	NC_002645.1	Complete	57%	52%
Human coronavirus OC43	NC_006213.1	Complete	No homology	No homology
Human coronavirus HKU1	NC_006577.2	Complete	No homology	No homology
Human coronavirus NL63	NC_005831.2	Complete	No homology	No homology
SARS-coronavirus	NC_004718.3	Complete	57%	52%
MERS-coronavirus	NC_019843.3	Complete	No homology	No homology
Human adenovirus A	NC_001460 .1	Complete	No homology	No homology
Human adenovirus B1	NC_011203.1	Complete	No homology	No homology
Human adenovirus B2	NC_011202.1	Complete	No homology	No homology
Human adenovirus C	NC_001405 .1	Complete	No homology	No homology
Human adenovirus 54	NC_012959.1	Complete	No homology	No homology
Human adenovirus E	NC_003266.2	Complete	No homology	No homology
Human metapneumovirus isolate 00-1	NC_039199.1	Complete	No homology	No homology
Parainfluenza virus 1	NC_003461.1	Complete	No homology	No homology
Parainfluenza virus 2	NC_003443.1	Complete	No homology	No homology
Parainfluenza virus 3	NC_001796.2	Complete	No homology	No homology
Parainfluenza virus 4a, Strain: M-25	NC_021928.1	Complete	No homology	No homology
Influenza A virus -A/New York/392/2004 (H3N2)	NC_007366.1-NC_007373.1	Complete	No homology	No homology
Influenza A virus - A/Puerto Rico/8/1934 (H1N1)	NC_002016.1-NC_002023.1	Complete	No homology	No homology
Influenza A virus - A/Korea/426/1968 (H2N2)	NC_007375.1-NC_007383.1	Complete	No homology	No homology
Influenza B virus-B/Lee/1940	NC_002204.1-NC002211.1	Complete	No homology	No homology
Human enterovirus 68 strain Fermon	NC_038308.1	Complete	No homology	No homology
Human enterovirus A	NC_001612.1	Complete	No homology	No homology
Human enterovirus B	NC_001472.1	Complete	No homology	No homology
Poliovirus	NC_002058 .3	Complete	No homology	No homology
Human enterovirus D	NC_001430.1	Complete	No homology	No homology
Respiratory syncytial virus	NC_001803.1	Complete	No homology	No homology
Human rhinovirus 14	NC_001490.1	Complete	No homology	No homology
Human rhinovirus 89	NC_001617.1	Complete	No homology	No homology
Human rhinovirus C	NC_009996.1	Complete	No homology	No homology
Chlamydia pneumoniae TW-183	NC_005043.1	Complete	No homology	No homology
Haemophilus influenzae strain NCTC8143	NZ_LN831035	Partial	No homology	No homology
Legionella pneumophila strain NCTC12273	NZ_LR134380.1	Partial	No homology	No homology
Mycobacterium tuberculosis H37Rv	NC_000962.3	Complete	No homology	No homology
Streptococcus pneumoniae strain NCTC7465	NZ_LN831051.1	Partial	No homology	No homology
Streptococcus pyogenes	NZ_LN831034	Partial	No homology	No homology

Pathogen Name	GenBank Accession ID	Genome	N1 Homology	N2 Homology
strain NCTC8189				
Bordetella pertussis 18323	NC_018518.1	Complete	No homology	No homology
Mycoplasma pneumoniae	NZ_CP010546.1	Partial	No homology	No homology
Pneumocystis jirovecii (PJP)	MK984200	Complete	No homology	No homology
Candida albicans strain L757	NC_018046	Complete	No homology	No homology
Pseudomonas aeruginosa PAO1	NC_002516.2	Complete	No homology	No homology
Staphylococcus epidermidis strain ATCC14990	NZ_CP035288.1-NZ_CP035290.1	Partial	No homology	No homology
Streptococcus salivarius strain NCTC8618	NZ_LR134274.1	Partial	No homology	No homology

3) *Clinical Evaluation:*

Performance of the Inform Diagnostics SARS-CoV-2 RT-PCR Assay was evaluated using clinical nasopharyngeal and oropharyngeal positive and negative swab specimens that were previously tested with the Inform Diagnostics' in-house unmodified CDC assay. A total of 31 confirmed negative patient samples and 33 confirmed positive patient samples were tested with the unmodified CDC assay. In addition, based on the reporting strategy for the original CDC EUA authorization, one result was indeterminate on both PCR platforms which was excluded from the performance analysis. Therefore, a total of 64 clinical specimens were used to assess the clinical performance of the Inform Diagnostics SARS-CoV-2 RT-PCR Assay (modified CDC assay) using both claimed PCR instruments including the ABI7500 Fast System and the QuantStudio.

For the 31 negative clinical NP and OP swab samples, the negative percent agreement (NPA) between the Inform Diagnostics' assay and the unmodified in-house CDC EUA assay used as the comparator was 100%. For the 33 clinical positive samples that were evaluated, 31/33 tested positive (93.9% PPA) using the Inform Diagnostics assay when run on the ABI7500 Fast System and 32/33 tested positive (96.9%) with the Inform Diagnostics assay when run on the QuantStudio. Qualitative results of the clinical evaluation are shown in Tables 5 and 6.

Table 5. Summary of Qualitative Clinical Study Results Performed on the ABI7500 Fast System

		Unmodified CDC EUA Authorized Assay - Comparator		
		Positive	**Negative**	**Total**
Inform Diagnostics SARS-CoV-2 RT-PCR Assay	**Positive**	31	0	31
	Negative	2	31	33
	Total	33	31	65
Positive Agreement		93.94% (31/33); 80.40-98.32% [1]		
Negative Agreement		100.00% (31/31); 88.65-100.00%		

[1]Two-sided 95% score confidence intervals

Table 6. Summary of Qualitative Clinical Study Results Performed on the QuantStudio Instrument

		Unmodified CDC EUA Authorized Assay - Comparator		
		Positive	**Negative**	**Total**
Inform Diagnostics SARS-CoV-2 RT-PCR Assay	**Positive**	32	0	32
	Negative	1	31	32
	Total	33	31	64
Positive Agreement		96.97% (32/33); 84.68-99.46% [1]		
Negative Agreement		100.00% (31/31); 88.65-100.00%		

[1]Two-sided 95% score confidence intervals

Discordant Analysis:

The discordant samples for the 3 false negative results generated between both platforms were investigated. It was determined that 2/3 discordant specimens produced late Ct values (~36 or higher) with the unmodified CDC assay, suggesting that these specimens may have had target levels below the LoD of the Inform Diagnostics SASR-CoV-2 RT-PCR Assay. For the third discordant, the investigation found that an insoluble pellet was present in the extracted RNA from this sample, which could have had an inhibitory effect on the RT-PCR process and subsequently led to the false negative result.

Additional Contrived Clinical Evaluation:

30 contrived positive samples were prepared by spiking synthetic viral RNA from Twist Bioscience into negatively screened nasopharyngeal swab matrix at 1.5X and 2X LoD. The clinical matrix used for spiking was screened negative using the unmodified CDC assay that Inform Diagnostics uses in-house. In addition, 30 negative clinical matrix samples were also tested. Samples were blinded and randomized for testing and RNA was extracted using the ViralXpress Reagent. Testing was performed in one RT-PCR run on both the ABI7500 and QuantStudio instruments with one positive, one negative, and one extraction control. Results of the study are summarized below (Table 7).

Table 7. Contrived Clinical Evaluation Summary Data

		ABI7500			QuantStudio		
SARS-CoV-2 concentration (copies/µL)	**Number of samples**	**Average Ct**		**Detection Rate**	**Average Ct**		**Detection Rate**
		N1	**N2**		**N1**	**N2**	
2X LoD (40 copies/µL)	15	27.83	33.70	15/15	32.43	36.57	15/15
1.5X LoD (30 copies/µL)	15	30.52	33.94	15/15	33.87	38.81	15/15
Negative	30	UND	UND	0/30	UND	UND	0/30

UND: Undetermined

The results at all tested levels demonstrated 100% agreement and all negative samples were non-reactive for all SARS-CoV-2 assay targets.

Clinical Confirmation:
In addition, the first 5 positive and 5 negative samples determined by the Inform Diagnostics SARS-CoV-2 RT-PCR Assay were sent to the Arizona Health Department running the CDC EUA test for confirmatory testing. All 10 patient specimens yielded concordant results.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.