ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay
Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard

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

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay will be performed at the Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard located at 320 Charles Street, Cambridge, Massachusetts 02141, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the laboratory procedure that was reviewed by the FDA under this EUA.

INTENDED USE

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs nasopharyngeal wash/aspirate or nasal aspirate specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their health care provider. Testing is limited to the Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard, located at 320 Charles Street, Cambridge, MA 02141 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 upper 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 CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay is a reverse transcriptase real-time polymerase chain reaction (rRT-PCR) assay for the qualitative detection of SARS-CoV-2 specific RNA. This test uses primer/probe sets developed by the CDC that target two viral gene targets in the Nucleocapsid gene of SARS-CoV-2, N1 and N2, and an internal control gene, RNase P (RP) for Assay Version 1 or N2 and RP for Assay Version 2.

The test consists of four processes in a single assay: 1) nucleic acid extraction, 2) reverse transcription of target RNA to cDNA, 3) PCR amplification of target and internal control DNA, and 4) simultaneous detection of PCR amplicons by fluorescent dye labelled probes.

There are two assay versions:

Assay Version 1 uses CDC developed SARS-CoV-2 nucleocapsid N1, N2, and human RP primers and probes. The respiratory specimen types are transported in VTM or sterile saline. Assay Version 1 was validated for use with the Applied Biosystems Viia7 thermocycler or the Applied Biosystems QuantStudio 7 Flex each with QuantStudio software version 1.3.

Assay Version 2 uses CDC SARS-CoV-2 nucleocapsid N2, and human RP primers and probes. The respiratory specimen types are transported in VTM or sterile saline. Assay Version 2 is validated for use with the Applied Biosystems QuantStudio 7 Flex thermocycler with QuantStudio software version 1.3.

Table 1. Summary of Similarities and Differences Between the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1 and Version 2.

	V1	**V2**
Transport medium	VTM or 0.9% sterile saline	VTM or 0.9% sterile saline
Liquid handler for initial sample handling	N/A	Hamilton STARlet
Extraction method	ThermoFisher MagMAX Viral RNA Isolation Kit	ThermoFisher MagMAX Viral RNA Isolation Kit
Extraction format	96-well	384-well
Liquid handler for extraction	Agilent Bravo	Agilent Bravo
Extraction sample input volume	50µl into extraction	37.5µl into extraction
Extraction output volume	50µl	15µl
Liquid handler for RT-PCR plate set-up	Formulatrix Tempest and Agilent Bravo	Formulatrix Tempest and Agilent Bravo
RT-PCR template volume	5µl	5µl
RT-PCR total reaction volume	15µl	10µl
RT-PCR plate format	384-well	384-well

	V1	V2
Thermocycler	Viia7 or QuantStudio 7 Flex	QuantStudio 7 Flex
Thermocycler software version	QuantStudio software version 1.3	QuantStudio software version 1.3

INSTRUMENTS USED WITH TEST

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay is to be used with the following instrumentation:

- Specimen Lysis/RNA Extraction/Realtime PCR Reagent Preparation: Agilent Bravo Liquid Handling Platform (running VWorks (Build 11.4.0.1233)) for automated extraction and/or RT-PCR plate set-up, and Formulatrix Tempest and Agilent Bravo Liquid Handling Platform also for RT-PCR plate set-up.
- RT-PCR Platforms: Applied Biosystems QuantStudio 7 Flex Real-Time PCR System (QuantStudio Software V1.3), Applied Biosystems ViiA7 (QuantStudio Software V1.3).

Table 2. REAGENTS AND MATERIALS

Item	Vendor	Vendor Catalog Number
4X TaqPath, 1-Step RT-qPCR Master Mix, GC (2,000 rxns/kit)	Thermo Fisher Scientific	A15300
2019-nCoV CDC EUA Primer/Probe Kit (500 rxns/box)	IDT	10006606
Tip, 1000ul Fltr, Conductive(480/PK)	Hamilton Robotics, Inc.	235905
Tip, 70ul, St. Fltr,Cond,Bravo(3840/PK)	AGILENT TECHNOLOGIES INC	19133-142
Plate, 384W, DW, V-Bottom(60/CA)	VWR International, LLC	82051-320
Plate, 384w Twintec Clear 40ul(25/BX)	VWR International, LLC	12000-658
Plate, 96w Twintec Clear (25/BX)	VWR International, LLC	47744-116
Plate, 384w Clear, FB, NS (100/CA)	VWR International, LLC	82051-298
AB-3720 Thermo foil pierce-able seal	Thermo Fisher Scientific	AB-3720
Ethanol, 100%, (24pints/CA)	VWR International, LLC	89125-170
Thermo Viral RNA Isolation Kit	Thermo Fisher Scientific	AMB18365
Isopropanol, 99% (4x4L/case)	VWR International, LLC	TXMK303216BRI
Wipe, RNase Zap Ambion (100/PK)	Thermo Fisher Scientific	AM9786
DNA ZAP!, Degradation Solution(250ml/BT)	Life Technologies, Inc.	AM9890
Disinfectant, Germicidal (625ml/BT)	WW GRAINGER CO	3VDL4
Ethanol, 70% USP 140Proof (32oz/BT)	VWR International, LLC	76212-358
Kit,2019-nCoV_N_Positive Control(1/bx)	Integrated DNA Technologies, Inc.	10006625
Tube, 50ml orange cap (25/PK)	VWR International, LLC	21008-775
Super rags (250/case)	BLUE THUNDER TECHNOLOGIES, INC.	WI-1318Q.250
Bags, Heavy weight, 8"x12" (100/pack)	VWR International, LLC	11215-280

Item	Vendor	Vendor Catalog Number
Tip, 2ml St. Fltr LTS(480/CA)	Mettler-Toledo Rainin LLC	17002923
Pipette, 25ml, Serological, St(200/CA)	VWR International, LLC	53392-198
Tip, pipette1000ul LTS Rainin (768/Ca)	Mettler-Toledo Rainin LLC	30389212
AB-3720 Thermo foil pierceable seal	Thermo Fisher Scientific	AB-3720
Kit, 2019-nCoV CDC EUA(500Rxn/BX)	Integrated DNA Technologies, Inc.	10006606
TaqPath,1Step RTqPCR MtrMx,CG(2000rx/KT)	Life Technologies, Inc.	A15300
Water, Sterile,Nuclease-Free (1000ml/BT)	VWR International, LLC	10220-384
DNA ZAP!, Degradation Solution(250ml/BT)	Life Technologies, Inc.	AM9890
Wipe, RNase Zap Ambion (100/PK)	Life Technologies, Inc.	AM9786

CONTROLS TO BE USED WITH THE CRSP SARS-CoV-2 REAL-TIME REVERSE TRANSCRIPTASE (RT)-PCR DIAGNOSTIC ASSAY

- A "no template" (negative) control (NTC) is used for every run and is needed to confirm that there is no contamination for the assay.

- A positive template control (COVID-19_N_Postive, IDT, #10006625) targeting the SARS-CoV-2 N-gene (N1 and N2) is used for every run and is needed to confirm that the assay is completed by the intended design.

- An internal control primer/probe set, targeting the human RNase P gene, is used for every patient sample to confirm appropriate specimen collection and to monitor the integrity of nucleic acid extraction and RT-PCR reactions.

- A human specimen (HSC) extraction control is included in each run to test for failure in lysis and extraction and potential contamination during 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 (Refer to **Table 3** for a summary of expected control results).

1. ***COVID-19 RT-PCR Test controls – Positive, Negative, Extraction, and Internal*:**

   Controls should produce the results outlined in **Table 3**, below.

Table 3. Expected Control Results for the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1*

Control Location	N1	N2	RP	Result Interpretation	Action
NTC well	-	-	-	Plate passes NTC QC	Plate sent for review and reporting

Control Location	N1	N2	RP	Result Interpretation	Action
NTC well	Any target positive			Plate Fails NTC QC	Plate reworked from RNA extraction
HSC well	-	-	+	Plate passes Extraction QC	Plate sent for review and reporting
HSC well	Any target positive		+/-	Plate fails Extraction QC	Plate reworked from RNA extraction
HSC well	-	-	-	Plate fails Extraction QC	Plate reworked from RNA extraction
nCoVPC	+	+	-	Plate passes Assay QC	Plate sent for review and reporting
nCoVPC	If ≤1 target is positive		-	Plate fails Assay QC	Plate reworked from RNA extraction

*Note: The results of the assay are reported according to the following categories where a + indicates a Ct of<40 and a - indicates a Ct of>40 or undetermined.

Table 4. Expected Control Results for the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 2.*

Control	N2	RP	Result Interpretation	Action
NTC wells (2 RP and 2 N2)	Both wells '-'	Both wells '-'	Plate passes NTC QC	Plate sent for review and reporting
NTC wells	Any well positive		Plate fails NTC QC	Plate reworked from RNA extraction
HSC+nCoVPC wells (2 RP and 2 N2)	≥1 out of 2 wells are '+'	≥1 out of 2 wells are '+'	Plate passes Extraction and Assay QC	Plate sent for review and reporting
HSC+nCoVPC wells (2 RP and 2 N2)	Both wells '-'	≥1 out of 2 wells are '+'	Plate fails Extraction and Assay QC	Plate reworked from RNA extraction
HSC+nCoVPC wells (2 RP and 2 N2)	≥1 out of 2 wells are '+'	Both wells '-'	Plate fails Extraction and Assay QC	Plate reworked from RNA extraction
HSC+nCoVPC wells (2 RP and 2 N2)	Both wells '-'	Both wells '-'	Plate fails Extraction and Assay QC	Plate reworked from RNA extraction

*Note: The results of the assay are reported according to the following categories where a + indicates a Ct of<40 and a - indicates a Ct of>40 or undetermined.

2. ***Examination and Interpretation of Patient Specimen Results***:
   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 **Tables 5 and 6** for guidance on patient specimen result interpretation and reporting of results.

Table 5. CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1*

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation
+	+	±	SARS-CoV-2 detected
If at least one target is positive		±	Inconclusive
-	-	+	SARS-CoV-2 not detected
-	-	-	Invalid Result

*Note: The results of the assay are reported according to the following categories where a + indicates a Ct of<40 and a - indicates a Ct of>40 or undetermined.

Table 6. CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 2*

2019 nCoV_N2	RP	Result Interpretation
+	+	SARS-CoV-2 detected
-	+	SARS-CoV-2 not detected
±	-	Invalid Result

*Note: The results of the assay are reported according to the following categories where a + indicates a Ct of<40 and a - indicates a Ct of>40 or undetermined.

PERFORMANCE EVALUATION

Analytical Sensitivity – Limit of Detection (LoD):

The LoD for Assay Version 1 SARS-CoV-2 detection was determined using dilutions of patient nasopharyngeal samples previously determined to be positive by the Massachusetts State Public Health Laboratory (MSPHL) CDC EUA assay. The SARS CoV-2 copy number in each patient sample was estimated using a relative standard curve generated by diluting SARS-CoV-2 synthetic RNA of a known concentration from Twist Biosciences (Cat no. MN908947.3, SKU: 102024). Each patient sample was diluted in VTM, independently extracted, and analyzed on the Applied Biosystems Viia7 thermocycler. Twenty of twenty replicates were detected at 4.0×10^3 copies/mL dilution (**Table 7**).

Table 7. CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1 LoD Confirmation Study.

Concentration	# Positive Replicates/Total # Replicates
4.0 x 10^3 copies/mL	20/20

The LoD for Assay Version 2 was determined using a dilution series of SARS-CoV-2 synthetic RNA of a known concentration from Twist Biosciences (Cat no. MN908947.3, SKU: 102024). Serial dilutions of the Twist synthetic RNA were created by spiking into the HSC controls, in VTM, pre-extraction, and carried through the RNA extraction and qRT-PCR using the Applied Biosystems QuantStudio 7 thermocycler using QuantStudio software version 1.3. The LoD was confirmed at 1.6 x 10^3 copies/mL as shown in **Table 8** below.

Table 8. CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 2 LoD Confirmation Study.

Concentration	# Positive Replicates/Total # Replicates	% Positive
2.7 x 10^3 copies/mL	19/20	95%
1.6 x 10^3 copies/mL	19/20	95%
1.1 x 10^3 copies/mL	18/20	90%
0.5 x 10^3 copies/mL	14/20	70%
0.3 x 10^3 copies/mL	13/20	65%
0.15 x 10^3 copies/mL	4/20	20%

Analytical Sensitivity – Inclusivity:

The sequences for the N1 and N2 primers/probes used in this assay are identical to the primer/probe sequences used in the FDA emergency use authorized CDC 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel. CDC has provided a right of reference to their Inclusivity Study data, which is available at https://www.fda.gov/media/134922/download.

Analytical Specificity – Cross-Reactivity:

In-silico Cross-Reactivity Assessment

The sequences for the N1 and N2 primers/probes used in this assay are identical to the primer/probe sequences used in the FDA emergency use authorized CDC 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel. CDC has provided a right of reference to their Cross-Reactivity Study data, which is available at https://www.fda.gov/media/134922/download.

Clinical Evaluation:

Performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1 was evaluated using the Applied Biosystems Viia7 thermocycler and residual specimens collected from individual patients. Thirteen positive oropharyngeal (OP) swabs, 10 positive nasopharyngeal (NP) swabs, 10 positive NP/OP swabs, and 40 negative NP swabs were all previously tested by Massachusetts State Public Health Laboratory (MSPHL) using the CDC EUA authorized SARS-CoV-2 test. The results from the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1 had 100% agreement with the expected results for the 73 samples compared to the EUA authorized SARS-CoV-2 comparator (**Table 9**).

Table 9. Summary of Clinical Performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 1 Compared to MSPHL CDC EUA Assay.

NP, OP, and NP/OP Specimens		**EUA Comparator Assay**		
		Positive	**Negative**	**Total**
CRSP SARS-CoV-2 Assay V1	**Positive**	33	0	33
	Negative	0	40	40
	Total	33	40	73
Positive Agreement		100% (33/33), 95% CI: (89.6, 100%)		
Negative Agreement		100% (40/40), 95% CI: (91.2, 100%)		

Performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 2 was evaluated using the Applied Biosystems QuantStudio 7 Flex (QuantStudio software version 1.3) using residual nasopharyngeal (NP) specimens collected in VTM from individual patients with results compared to Assay Version 1. There was 100% positive and negative agreement for Assay Version 2 compared to Assay Version 1 (**Table 10**).

Table 10. Summary of Clinical Performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 2 Compared to Version 1 With NP Specimens in VTM.

NP Swab Specimens		**CRSP SARS-CoV-2 Assay Version 1**		
		Positive	**Negative**	**Total**
CRSP SARS-CoV-2 Assay Version 2	**Positive**	30	0	30
	Negative	0	28	28
	Total	30	28	58
Positive Agreement		100% (30/30), 95% CI: (88.7, 100%)		
Negative Agreement		100% (28/28), 95% CI: (87.9, 100%)		

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to Clinical Research Sequencing Platform, LLC at the Broad Institute of MIT and Harvard, Cambridge, MA 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. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- The performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay was established using nasopharyngeal (NP), oropharyngeal (OP), and combined nasopharyngeal/oropharyngeal (NP/OP) swabs in viral transport media (VTM) or 0.9% sterile saline. Nasal swabs (in VTM or 0.9% sterile saline), mid-turbinate nasal swabs (in VTM or 0.9% sterile saline), anterior nasal swabs (in VTM or 0.9% sterile saline), nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage specimens are also considered acceptable specimen types for use with the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay, but the performance has not been established with these specimens.
- Dry nasal swabs, dry anterior nasal swabs, and dry mid-turbinate nasal swab specimens have not been validated for use with the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay. Additional studies are required to determine suitability of dry nasal swabs, dry anterior nasal swabs, and dry mid-turbinate nasal swabs for use with the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

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 laboratories;
- 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.