EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3)
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 (Version 3) 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, and meets requirements to perform high complexity tests, as described in the standard operating 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 (Version 3) is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in dry anterior nasal swabs from individuals suspected of COVID-19 by their healthcare provider.

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) is also authorized for the qualitative detection of nucleic acid from SARS-CoV-2 in dry anterior nasal swab specimens collected using the CRSP Self-Swab kit, by individuals 18 years of age and older (self-collected), 14 years and older (self-collected under adult supervision), or 2 years and older (collected with adult assistance) suspected of COVID-19 by their healthcare provider and when determined to be appropriate by the healthcare provider. The kit is provided to individuals by the healthcare provider and the specimens collected using the CRSP Self-Swab kit will be dropped off at the designated location and transported via courier for testing at the Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard.

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) is also authorized for use with anterior nasal swab specimens collected using either: (1) the Color COVID-19 Self-Swab Collection Kit when used consistent with its authorization; or (2) the binx Health At-home Nasal Swab COVID-19 Sample Collection Kit when used consistent with its authorization.

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version3) is also authorized for qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 10 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID- 19, when tested as part of a serial testing program including testing at least once per week.

All 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 anterior nasal 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 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 (Version 3) 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 (Version 3) is a multiplexed, 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) in a multiplexed reaction.

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

INSTRUMENTS USED WITH THE TEST

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) is to be used with the following instrumentation:

Specimen Rehydration/Transfer/Lysis/RNA Extraction/Real-time PCR Reagent Preparation: For individual diagnostic tests, a Neutec (TF3000) tube and bottle filler is used to add rehydration buffer to dry anterior nasal specimens in empty (i.e., no transport media) swab tubes. For pooled tests, a TekMill automated decapper and filler is used to add rehydration buffer to dry anterior nasal specimens containing between 1 and 10 swabs. A

Hamilton STARlet automatic liquid handler is then used to transfer rehydrated patient or pooled specimens from swab tubes to 96 well microtiter plates. 4 discrete 96 well microtiter plates are consolidated into a 384 well microtiter plate using a Dynamic Devices Lynx LM1200 Liquid handler. An Agilent Bravo Liquid Handling Platform (running VWorks (Build 11.4.0.1233)) is implemented for automated extraction and/or RT-PCR plate set-up.

- **RT-PCR Platform:** Applied Biosystems QuantStudio 7 Flex Real-Time PCR System (QuantStudio Software V1.3)

REAGENTS AND MATERIALS

Table 1. Reagents and Materials

Reagent/Material	Vendor	Vendor Catalog Number
MagMAX-96 Viral RNA Isolation Kits	Thermo Fisher Scientific	AMB18365
Kit,2019-nCoV_N_Positive Control (1/bx)	Integrated DNA Technologies, Inc.	10006625
Rehydration/Lysis Solution (20L)	SIGMA-ALDRICH Inc	L8285-CONF
Chemagic Viral Lysis Buffer	Perkin Elmer	CMG-832
Swab Nucleic Acid Preservative (1L)	Norgen Biotek	68800-1L
nCOV_N1 Forward Primer Aliquot, 100 nmol	Integrated DNA Technologies, Inc.	10006830
nCOV_N1 Reverse Primer Aliquot, 100 nmol	Integrated DNA Technologies, Inc.	10006831
nCOV_N1(FAM) Probe Aliquot, 50 nmol	Integrated DNA Technologies, Inc.	10006832
nCOV_N2 Forward Primer Aliquot, 100 nmol	Integrated DNA Technologies, Inc.	10006833
nCOV_N2 Reverse Primer Aliquot, 100 nmol	Integrated DNA Technologies, Inc.	10006834
nCOV_N2 (SUN) Probe, 50 nmol	Integrated DNA Technologies, Inc.	10007049
RNase P Forward Primer Aliquot, 100 nmol	Integrated DNA Technologies, Inc.	10006836
RNase P Reverse Primer Aliquot, 100 nmol	Integrated DNA Technologies, Inc.	10006837
RNase P (ATTO 647) Probe, 50 nmol	Integrated DNA Technologies, Inc.	10007062
TaqPath,1Step RTqPCR MtrMx,CG(2000rx/KT)	Life Technologies, Inc.	A15300

CONTROLS TO BE USED WITH THE TEST

All controls are included with each run of the assay. On each 384 well qPCR detection plate there are four Negative Sample Control (NSC) wells and four Positive Template Control (PTC) wells. An internal control for RNase P is run with each patient specimen.

1. **Negative Sample Control (NSC)**: Cultured A549 human cell line material suspended in 0.01M PBS at pH 7.2-7.4 with ~10,000 cells per input aliquot.
2. **Positive Template Control (PTC)**: Cultured A549 human cell line material suspended in 0.01M PBS at pH 7.2-7.4 with ~10,000 cells per input aliquot with addition of 2019-nCoV_N_Positive Control, IDT (Cat no. 10006625), 8000 copies/mL.
3. **Internal Control**: A primer and probe set for RNase P is included that amplifies the human RNAse P gene in the dry anterior nasal swab specimen.

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 2** for a summary of expected control results).

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

Control Location	N1	N2	RP	Result Interpretation	Action
NSC wells	All wells '-'	All wells '-'	≥ 1 out of 4 wells are '+'	Plate passes NSC QC	Plate sent for review and reporting
NSC wells	Any well '+'		≥ 1 out of 4 wells are '+'	Plate fails NSC QC	Plate reworked from RNA extraction
PTC wells	At least 1 well '+'	At least 1 well '+'	≥ 1 out of 4 wells are '+'	Plate passes PTC QC	Plate sent for review and reporting
PTC wells	All wells '-'	All wells '-'	±	Plate fails PTC QC	Plate reworked from RNA extraction

*In the probe columns, '+' indicates a Ct <40 and '-' no detectable signal ('Undetermined' in the QuantStudio software). Both NSC and PTC QCs must pass for a plate to pass QC.

Assessment of clinical specimen test results is performed after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted. Please see **Table 3** for guidance on patient specimen result interpretation and reporting of results.

The results of the assay are reported according to the following categories where a '+' indicates a Ct of <40 and a '-' indicates the absence of a Ct <40. a '±' indicates either a Ct value or no Ct value (Table 3):

Table 3. CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3)*

2019 nCoV_N1	*2019 nCoV_N2*	*RP*	*Result Interpretation*	*Individual Test Action*	*Pooled Test Action*
+	+	±	*SARS-CoV-2 detected*	*Report results to the healthcare provider and appropriate public health authorities.*	*Report results to the testing site and direct the site to follow the deconvolution testing protocol[3].*
If only one target is '+'		±	*Inconclusive[1,2]*	Report results to the healthcare provider and recommend collection of a new specimen.	*Report results to the testing site and direct the site recollect specimens from the individuals in the pool.*
-	-	+	*SARS-CoV-2 not detected*	*Report results to the healthcare provider and appropriate public health authorities.*	*Report results to the testing site.*
-	-	-	*Invalid Result*	Report results to the healthcare provider and recommend collection of a new specimen.	*Report results to the testing site and direct the site recollect specimens from the individuals in the pool.*

[1] Recommend that a new specimen is collected and tested.

[2] To monitor for emergence of any SARS-CoV-2 mutations that may impact the performance of either of the viral probes, specimens in which only one probe is detected at a Ct of ≤30 will be flagged. These specimens will be retrieved and sent for surveillance sequencing via NGS at Broad.

[3] For pooled swab testing, when SARS-CoV-2 is detected, the result is delivered as "presumed positive". Sites with a pool in which SARS-CoV-2 is detected are directed to retest the individuals in the pool with a diagnostic test – either the Rapid Antigen BinaxNOW test or an individual RT-PCR test. Sites are provided text to indicate that such individuals should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result. Results are also delivered with text that indicates that "Individual specimens with low viral loads may not be detected due to the decreased sensitivity or increased interference when tested with pooled testing."

PERFORMANCE EVALUATION

Analytical Sensitivity -Limit of Detection (LoD)

The limit of detection (LoD) is defined as the lowest concentration at which 19/20 replicates (or approximately 95% of all true positive replicates) are positively detected. The LoD of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) was established using a dilution series from a SARS-CoV-2 N gene synthetic construct of known titer

from Twist Biosciences (Cat no. MN908947.3, SKU: 102024). A dilution series from 1 million copies to 1 copy were generated in negative clinical matrix from confirmed negative patient anterior nasal swabs. The dilutions proceeded through extraction and were tested in the rRT-PCR assay. The preliminary LoD was determined to be 10 copies/rxn.

The LoD was confirmed by testing a natural clinical specimen with a viral load calculated from extrapolation of the Ct value against a standard curve generated with a SARS-CoV-2 N gene synthetic construct of known titer from Twist Biosciences (Cat no. MN908947.3, SKU: 102024). The natural clinical specimen was diluted by adding the specimen to rehydration/lysis buffer from Sigma (Sigma-Aldrich Inc., L8285-CONF) or lysis buffer from the Chemagic Viral DNA/RNA kit (Perkin Elmer, CMG-1033 (Lysis buffer component, CMG-832)), and then pipetting the diluted sample material onto dry swabs. The swabs were allowed to dry before testing with the entire workflow. The final LoD was determined to be 60 copies/rxn (1600 copies/mL) as that was the lowest dilution at which 20/20 replicates were positive. Results for the Sigma-Aldrich rehydration buffer are shown in **Table 4**, while the results for the Chemagic buffer are shown in **Table 5**.

Table 4. Summary of Confirmatory LoD Study Results – Sigma-Aldrich Rehydration Buffer

Copies/mL	# Detected/ # Tested by Target Gene			Mean Ct (Std Dev)		
	N1	N2	RNase P	N1	N2	RNase P
1600	100% (20/20)	100% (20/20)	100% (20/20)	32.6 (0.44)	35.8 (0.8)	31.8 (0.3)

Table 5. Summary of Confirmatory LoD Study Results – Chemagic Rehydration Buffer

Copies/mL	# Detected/ # Tested by Target Gene			Mean Ct (Std Dev)		
	N1	N1	N2	N1	N2	RNase P
1600	100% (20/20)	100% (20/20)	100% (20/20)	32.5 (0.7)	35.5 (0.7)	32.3 (0.9)

Analytical Sensitivity – Inclusivity

In-silico Inclusivity 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 Inclusivity Study data, which is available at https://www.fda.gov/media/134922/download

In addition, CRSP performed an alignment with the oligonucleotide primer and probe sequences for the N1 and N2 targets with all publicly available, U.S. nucleic acid sequences for SARS-CoV-2 in GenBank as of Feb 9, 2021 and GISAID as of Feb 18, 2021. After filtering for duplicates and low quality or incomplete sequences, 119,649 complete (across the N gene regions), high quality sequences were reviewed (30,899 from GenBank and 88,750 from GISAID). A software package (openPrimeR[1]) was used to calculate primer binding efficiency. This measure takes into consideration the primer sequence, the template sequence, the reaction conditions, and the location of any mismatches between primer/probe and template. A total of 6 submitted sequences (0.005% of all reviewed sequences) were identified (representing 9 unique

specimens) where the N2 primer (forward or reverse) or probe binding efficiency were estimated to be reduced). Similarly, a total of 29 submitted sequences (0.02% of all reviewed sequences) (from 44 unique specimens) predicted to have decreased N1 primer/probe binding efficiency. Notably, there were no submitted sequences identified where both N1 and N2 primers/probes were predicted to demonstrate decreased binding efficiency.

[1]https://bioconductor.org/packages/release/bioc/html/openPrimeR.html

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 Assay (Version 3) was evaluated using remnant dry anterior nasal swab specimens collected from individuals suspected of COVID-19. These specimens were tested with the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) and an EUA authorized high-sensitivity SARS-CoV-2 test. Fifty-nine (59) specimens were positive and 109 specimens were negative by the EUA authorized SARS-CoV-2 comparator assay. Positive agreement and Negative agreement were 98.3% and 100%, respectively. The results of the study are illustrated in **Table 6**.

Table 6. Summary of Clinical Performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) Compared to an EUA Authorized Comparator Assay

Dry Anterior Nares Swab Specimens		**EUA Authorized High Sensitivity Comparator**		
		Positive	**Negative**	**Total**
CRSP SARS-CoV-2 Assay (Version 3)	**Positive**	58	0	58
	Negative	1	109	110
	Total	59	109	168
Positive Agreement		98.3% (58/59), 95% CI: (91.0, 99.7%)		
Negative Agreement		100.0% (109/109), 95% CI: (96.6, 100%)		

CRSP Self-Swab Kit Validation Data

The CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) is a modified version of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay Version 2, which was previously authorized for at-home collection with the CRSP Self-Swab Kit. The CRSP SARS-CoV-2 Assay (Version 3) is identical to assay Version 2, except that the primers and probes have been multiplexed in Version 3. Validation data for the CRSP Self-Swab Kit is described in the following sections.

Human Usability Study for CRSP Self-Swab Kit

CRSP performed two usability studies for unsupervised collection using the CRSP Self-Swab Kit and dropping the sample off for transport to the CRSP CLIA-certified lab for testing. The Collection Instructions were updated based on the first study results and prior to conducting the second study. Actual-use testing was performed with 30 participants. The site observers observed the participant using the collection kit at a simulated kit swab/drop-off site. Cognitive debriefing interviews were conducted following the actual-use testing to gather users' perspectives on each critical task or use scenario and answers to survey questions were collected. Results of the usability testing were analyzed qualitatively to determine if the design of the kit and/or kit instructions need to be modified to reduce the use-related risks to acceptable levels.

Successful sample collection was measured by the presence of human RNase P in the sample. All samples were acceptable for testing and no samples failed processing due to particulate matter. The RNase P was detected in all samples (30/30).

CRSP Self-Swab Kit Stability

The CRSP Self-Swab Kit uses the same dry spun polyester swabs as the binx health At-home Nasal Swab COVID-19 Sample Collection Kit and binx health has granted a right of reference to CRSP, LLC at The Broad Institute of MIT and Harvard for sample stability validation data generated by binx health. Therefore, these data support the use of the CRSP Self-Swab Kit for ambient temperature transport and storage of anterior nasal swab specimens collected at home for up to 120 hours from the time of collection.

CRSP Self-Swab Kit Pediatric Use

The CRSP Self-Swab Kit uses the same dry spun polyester swabs as the Color COVID-19 Self-Swab Collection Kit. Color Health, Inc. has granted a right of reference to CRSP, LLC at The Broad Institute of MIT and Harvard for usability validation data generated by Color Health, Inc. Therefore, these data support the use of the CRSP Self-Swab Kit with specimens collected from patients including those age 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 2 years and older (collected with adult assistance).

Additional Home Collection Kits Authorized for Use with this Assay

This assay can be used with the Color COVID-19 Self-Swab Collection Kit. Color Health, Inc. has granted Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard a right of reference to the data supporting use of this collection kit.

This assay can be used with the binx Health At-home Nasal Swab COVID-19 Sample Collection Kit. Binx health has granted Clinical Research Sequencing Platform (CRSP), LLC at the Broad Instituteof MIT and Harvard a right of reference to the data supporting use of this collection kit**.**

LIMITATIONS:

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to the Clinical Research Sequencing Platform, 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. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may resultin erroneous results

- The performance of the CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay (Version 3) was established using "dry" anterior nasal swabs in a sterile tube (with no transport medium).
- 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.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The following indication is authorized under the Pooling and Serial Testing Amendment [https://www.fda.gov/media/147737/download] for use in the 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 for the Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 10 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested as part of a serial testing program including testing at least once per week.
  This indication is authorized with the following validated protocol: pooling of up to 10 individual human anterior nasal swabs.

WARNINGS:

- For use under Emergency Use Authorization (EUA) only.
- For *in vitro* diagnostic use.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized foremergency use by FDA under an EUA for use by the authorized laboratory.
- This product has been authorized only for the detection of nucleic acids from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declarationthat 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 theFederal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.