ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY CDI ENHANCED COVID-19 TEST (HACKENSACK UNIVERSITY MEDICAL CENTER, MOLECULAR PATHOLOGY LABORATORY)

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

(The CDI Enhanced COVID-19 Test will be performed at the Molecular Pathology Laboratory at Hackensack University Medical Center, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The CDI Enhanced COVID-19 Test 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 aspirate specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Hackensack University Medical Center (HUMC) Molecular Pathology Laboratory located in Hackensack, NJ which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, certified high-complexity laboratory.

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 CDI Enhanced COVID-19 Test is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The CDI Enhanced COVID-19 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Testing of self-collected or healthcare provider-collected anterior and mid-turbinate nasal swabs is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The CDI Enhanced COVID-19 Test is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The test uses one primer and probe set to detect one region in the SARS-CoV-2 nucleocapsid (N) gene (N2), one primer and probe set for the universal detection of SARS-like coronaviruses (E), and one primer and probe set to detect human RNase P (RP) in a clinical sample.

RNA is isolated from upper respiratory specimens including nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens using the Roche MagNA Pure 24 System and is reverse transcribed to cDNA and subsequently amplified using the Mic PCR cycler from Bio Molecular System with the software micPCR v2.8.0 or v2.8.13. 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 CDI Enhanced COVID-19 Test is to be used with the MagNA Pure 24 System and the Mic PCR cycler with micPCR software version2.8.0 or version2.8.13.

REAGENTS AND MATERIALS

Reagent Manufacturer and Description	Catalog #	Manufacturer
MagNA Pure 24 System	07290519001	Roche Molecular Systems, Inc.
One Step PrimeScript RT-PCR Kit (Perfect Real Time)	RR064B/RR064A	Takara Bio USA, Inc.
SensiFAST Probe No-ROX One-Step Kit	BIO-76005/76001	Bioline
COVID-19 N2-F Primer (forward primer)	0000507509	Integrated DNA Technologies
COVID-19 N2-R Primer (reverse primer)	0000507509	Integrated DNA Technologies
COVID-19 N2-P Probe (N2 probe)	0000507509	Integrated DNA Technologies
E-F	Custom synthesized	Integrated DNA Technologies
E-R	Custom synthesized	Integrated DNA Technologies
E-P	Custom synthesized	Integrated DNA Technologies
RP-F Primer (forward primer)	0000508785	Integrated DNA Technologies
RP-R Primer (reverse primer)	0000508785	Integrated DNA Technologies
RP-P Probe (RNase P probe)	0000508785	Integrated DNA Technologies
nCoVPC (Positive RNA control)	NR-52285	BEI Resources
RPPC (DNA positive control)	7038-Pos	ScienCell
Human Specimen Control	7038-Hsc	ScienCell
BD Universal Viral Transport (UVT) 3-mL collection kit with regular and flexible minitip flocked swab	220527	Becton Dickinson

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

1) A "no template" (negative) control (NTC) is needed to check for contamination of extraction and 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) Two positive controls are used to verify proper assay set-up and SARS-CoV-2 reagent integrity. Viral genomic RNA acquired from BEI resources (Cat #NR-52285) is used as positive control (nCoVPC) for the rRT-PCR N2 and E assays. The DNA positive control is purchased from ScienCell (Cat #7038-Pos) and is used as a positive control (RPPC) for the RP assay. The RPPC 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.

3) The human specimen extraction control (HSC) is a human RNA extract from non-infected samples that is purchased from ScienCell (Cat #7038-Hsc). 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. The HSC is used in each batch of extractions.

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 1 for a summary of control results).

1) COVID-19 RT-PCR test Controls – Positive, Negative, and Internal:

- All NTC reactions should be negative for all targets, meaning no amplification curves cross the PCR cycle threshold (Ct). If any of the N2, E, or RP NTC reactions 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. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is positive, re-extract and re-test all samples.

- Positive control reactions for the N2 and E assays should yield positive results with a Ct value < 40.0. Because the positive control also contains the DNA positive control (RPPC) for the RP target, the RNase P target should also be positive (Ct < 40). Negative results with either N2 or E primer/probe sets invalidates the run and suggests the assay may have been set up incorrectly, or the integrity of the primers/probes is compromised. Repeat from the RT-PCR step using residual extraction material.

- RNase P (RP) reactions should yield negative results with the N2 and E assays, and a positive result on the RP assay with a Ct value < 40. Failure of a patient sample to yield an RP Ct value < 40 may indicate improper extraction of nucleic acid from patient samples, carry-over of PCR inhibitors from patient samples, or absence of sufficient human cellular material. Re-extract the residual sample and HSC and re-test the sample.

Table 1: Expected Results of Controls Used in the CDI Enhanced COVID-19 Test

Control Type	External Control Name	Used to Monitor	CDI SARS-like CoV E-gene	CDI SARS-CoV-2 N2-gene	RP	Expected Ct Values
Positive	nCoVPC+ RPPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00
Negative	NTC	Reagent and/or environmental contamination	-	-	-	Not detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40.00

2) <u>Examination and Interpretation of Patient Specimen Results:</u>

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 CDI Enhanced COVID-19 Test

SARS- CoV-2 (N2 gene) Ct < 40	SARS-like coronaviruses (E gene) 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 is **Presumptively Positive**	**PRESUMED POSITIVE**	Sample is repeated once on extracted RNA. If the repeated result remains "PRESUMPTIVE POSITIVE", additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Reported to sender. Consider testing for other respiratory viruses.

-	-	-	**Invalid Result**	**INVALID**	Repeat extraction and RT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient, if clinically indicated.

PERFORMANCE EVALUATION

1) *Analytical Sensitivity:*

Limit of Detection (LoD):

The LoD of the CDI Enhanced COVID-19 Test was determined using quantified whole viral SARS-related coronavirus 2 (USA-WA1/2020) RNA obtained from BEI Resources (NR-52285). A preliminary LoD was determined by testing serial dilutions (2400 copies/rxn - 3 copies/rxn) of RNA spiked into pooled clinical negative, nasopharyngeal swab matrix in triplicate. Spiked samples were tested with the CDI Enhanced COVID-19 Test following extraction with the MagNA Pure 24 instrument. Real-Time RT-PCR assays were performed using the One Step PrimeScript RT-PCR Kit (Perfect Real Time) (Takara) and the SensiFAST Probe No-ROX One-Step Kit (Bioline) on the Bio Molecular Systems Mic qPCR cycler.

The preliminary LoD study results showed 3/3 positives at all levels tested including 3 copies/rxn when using the One Step PrimeScript RT-PCR Kit. The initial LoD determination of the CDI Enhanced COVID-19 Test was 10 copies/rxn (4 copies/µL) when using the SensiFAST Probe No-ROX One-Step Kit.

The LoD was verified by testing at least 20 additional extraction replicates consisting of pooled negative clinical nasopharyngeal swab matrix spiked at 4 copies/µL (20 copies/rxn) and 2 copies/µL (10 copies/rxn). Samples were spiked with RNA prior to extraction with the MagNA Pure 24 instrument. The LoD of the CDI Enhanced COVID-19 Test was confirmed with both the Takara and Bioline RT-PCR Kits.

The assay failed with 2 copies/µL (10 copies/rxn) when using the Takara master-mix. Therefore, the established LoD for both the E and N2 assays of the CDI Enhanced COVID-19 Test was 4 copies/µL (20 copies/rxn) with the Takara RT-PCR kit (Table 3).

An additional lower concentration (1 RNA copy/µL; 5 copies/rxn) was also tested in the LoD confirmation study as 2 copies/µL (10 copies/rxn) for both the E and N2 targets generated 22/22 results when using the Bioline RT-PCR Kit. Table 4 shows that the LoD of the CDI Enhanced COVID-19 Test was 2 RNA copies/µL with the Bioline master-mix. However, during the clinical evaluation, the LoD of 2 copies/µL (10 copies/rxn) failed and therefore, the LoD of 4 RNA copies/µL (20 copies/rxn) for both the E and N2 targets using the Bioline kit was established.

The results of the LoD confirmatory study are summarized below in Tables 3-4.

Table 3: LoD Verification Study Results Using the Takara One Step PrimeScript RT-PCR Kit

Targets	SARS-like coronaviruses (E gene)		SARS-CoV-2 (N2 gene)	
Concentration (copies/µL)	4	2	4	2
Concentration (copies/reaction)	20	10	20	10
Positives/Total	20/20	12/20	19/20	17/20
Mean Ct[a]	33.2	NA	35.0	NA
Standard Deviation (Ct)	0.8	NA	0.9	NA

[a] Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

Table 4: LoD Verification Study Results Using the Bioline SensiFAST Probe N-ROX One-Step Kit

Targets	SARS-like coronaviruses (E gene)			SARS-CoV-2 (N2 gene)		
Concentration (copies/µL)	4	2	1	4	2	1
Concentration (copies/reaction)	20	10	5	20	10	5
Positives/Total	24/24	22/22	20/24	24/24	22/22	23/24
Mean Ct[a]	33.8	34.4	NA	36.1	36.8	37.8
Standard Deviation (Ct)	0.4	0.7	NA	0.9	1.2	0.8

[a] Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

2) ***Analytical Inclusivity/Specificity:***

In Silico Analysis of Primer and Probe Inclusivity/Exclusivity for N2 Target:

The CDI Enhanced COVID-19 test utilizes the identical oligonucleotide sequences for the N2 and RP genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. In silico testing of the SARS-CoV-2 N2 assay was previously performed by CDC as part of their EUA authorized test. The inclusivity and cross-reactivity of the CDC EUA assay has been previously evaluated and therefore, additional evaluation for the N2 target is not required. 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.

In Silico Analysis of Primer and Probe Inclusivity for E Target:

For the E assay, an alignment (as of March 10, 2020) of the E primers and probe was performed against a total of 7849 genome sequences of *betacoronavirus*, including 488 sequences of SARS-CoV-2 (n=96), SARS-CoV, and bat SARS-CoV. The forward primer had 100% homology with sequences from all SARS-CoV-2 genomes and both human and bat SARS-CoV. One SARS-CoV sequence (GenBank Accession number EU371564) had a homology of 96.1% to the forward primer of the E assay. The reverse E primer also had 100% homology with all SARS-like coronaviruses in the alignment except one SARS-CoV sequence (GenBank Accession number

FJ882960) had one mismatch with 95.5% homology. The E probe was found to be 100% identical with the sequences from all SARS-CoV-2 as well as the majority of sequences from human and bat SARS-CoV. Six human SARS-CoV (GenBank Accession numbers are KP886809, AY559085, AY559095, EF199652, AY536758, KY352407) had 1~3 mismatches in the probe sequence (identity ranges from 88% to 96%), and two bat SARS-CoV had homology of 96% (GenBank Accession number KP886809) and 84% (GenBank Accession number GQ153543) with the E probe, respectively. Sequences for both E primers and probe were also blasted against all available human genome sequences, other coronaviruses or respiratory human pathogens as listed in the cross-reactivity section. No significant homology with any of these sequences was observed that would predict potential false positive rRT-PCR results.

In Silico Analysis of Primer and Probe Cross-Reactivity for E Target:
BLASTn analysis queries of the SARS-CoV-2 rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank + EMBL + DDBJ + PDB + RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 03/10/2020; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

The N2 assay has <80% homology to the organisms listed in Table 5. The E assay has 88~100% homology with human SARS-CoV and 84~100% homology with bat SARS coronavirus, but <80% homology with all other organisms listed in the Table below.

Table 5: Organisms Assessed In Silico for Potential Cross-Reactivity

Viruses	**Bacteria/Candida**
Human coronavirus 229E	*Chlamydia pneumoniae*
Human coronavirus OC43	*Haemophilus influenzae*
Human coronavirus HKUl	*Legionella pneumophila*
Human coronavirus NL63	*Mycobacterium tuberculosis*
SARS-coronavirus	*Streptococcus pneumoniae*
MERS-coronavirus	*Streptococcus pyogenes*
Adenovirus (e.g. Cl Ad.71)	*Bordetella pertussis*
Human Metapneumovirus	*Mycoplasma pneumoniae*
Parainfluenza virus 1-4	*Pneumocystis jirovecii* (PJP)
Influenza A & B	Pooled human nasal wash -to represent diverse microbial flora in the human respiratory tract
Enterovirus (e.g. EV68)	*Candida albicans*
Respiratory syncytial virus	*Pseudomonas aeruginosa*

Viruses	Bacteria/Candida
Rhinovirus	*Staphylococcus epidermidis*
	Staphylococcus salivarius

3) Clinical Evaluation:

Performance of the CDI Enhanced COVID-19 Test was evaluated using individual clinical nasopharyngeal swab specimens that were previously tested by the BioFire FilmArray Respiratory Panel. Of these clinical specimens, 20 were positive for an organism on the BioFire Respiratory Panel and 10 were negative by the panel. Another 30 contrived specimens were spiked with viral genomic RNA from BEI Resources to produce the following viral load: 20 (or 21 samples) with 2X LoD and 10 (or 9 samples) additional samples ranging from 3X to 4X LoD as shown below in Tables 6-7.

These 60 samples (30 spiked positives, 30 clinical negative samples) were randomized and blinded, and RNA was extracted using the MagNA Pure 24 system. Testing was performed in one RT-PCR run with one positive, one negative, and one extraction control included per plate. Each sample also contained its own internal extraction control. Results of the study using the two different master mixes are summarized below in Tables 6-7.

Table 6: Clinical Evaluation Summary Data using the TaKara One Step PrimeScript RT-PCR Master Mix (LoD – 4 copies/uL; 20 copies/rxn)

Concentration (copies/rxn)	Number of samples	Average Ct			Detection Rate		
		E	N2	RP	E	N2	RP
2X LoD (40 copies/rxn)	20	33.3	35.1	27.1	20/20	20/20	20/20
3X LoD (60 copies/rxn)	5	31.6	34.3	27.5	5/5	5/5	5/5
4X LoD (80 copies/rxn)	5	32.1	33.6	27.3	5/5	5/5	5/5
Negative Clinical Samples (no copies)	30	ND	ND	ND	0/30	0/30	30/30

Table 7: Clinical Evaluation Summary Data using the Bioline SensiFAST Probe No-ROX One-Step Kit (LoD – 4 copies/µL; 20 copies/rxn)

Concentration (copies/rxn)	Number of samples	Average Ct			Detection Rate		
		E	N2	RP	E	N2	RP
2X LoD (40 copies/rxn)	21	34.8	34.4	28.2	21/21	21/21	21/21
3X LoD (60 copies/rxn)	5	34.8	33.5	26.9	5/5	5/5	5/5
4X LoD (80 copies/rxn)	4	34.2	32.8	27.5	4/4	4/4	4/4

Concentration (copies/rxn)	Number of samples	Average Ct			Detection Rate		
		E	N2	RP	E	N2	RP
Negative Clinical Samples (no copies)	30	ND	ND	ND	0/30	0/30	30/30

The results at all tested levels for spiked positives in clinical matrix demonstrated 100% agreement and all negative samples were non-reactive.

The testing on these clinical specimens performed at Hackensack University Medical Center and at the alternate testing laboratory fulfills the requirement for confirmatory testing for at least five positive and five negative specimens.