ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
The TRIPLEX CII-SARS-CoV-2 rRT-PCR TEST
(Columbia University Laboratory of Personalized Genomic Medicine)

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

(The Triplex CII-SARS-CoV-2 rRT-PCR test will be performed at the Columbia University Laboratory of Personalized Genomic Medicine in New York, NY, 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 Triplex CII-SARS-CoV-2 rRT-PCR test 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, oropharyngeal, nasal, and mid-turbinate nasal swab samples collected from individuals suspected of COVID-19 by their healthcare provider. 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. The SARS-Co-V-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infections. 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. Testing is limited to Columbia University Laboratory of Personalized Genomic Medicine, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests.

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 Triplex CII-SARS-CoV-2 rRT-PCR test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Triplex CII-SARS-CoV-2 rRT-PCR test is only for use under the Food and Drug Administration's Emergency Use Authorization.

LIMITATIONS

For *in vitro* diagnostic use only.
For prescription use only.
For use under Emergency Use Authorization (EUA) only.

Based on the *in silico* analysis, SARS-CoV-1 may cross-react with the Triplex CII-SARS-CoV-2 rRT-PCR test. SARS-CoV-1 is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Nucleic acids are isolated and purified from patient sample collected in VTM using bioMérieux NucliSENS easyMag system (250 µL sample input, 750 µL lysis buffer, 50 µL elution volume) or the QIAcube automated extraction platform (200 µL sample input, 200 µL lysis buffer, 100 µL elution volume). 5 µL of the purified nucleic acid is reverse transcribed, using the RNA UltraSense One-Step Quantitative RT-PCR System (ThermoFisher Scientific, catalog # 11732927), into cDNA, which is then subsequently amplified in the ABI Fast. 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 DNA Polymerase degrades the bound probe, causing the reporter dye (FAM or Quasar 670) to separate from the quencher dye (BHQ1 or BHQ-3 plus), generating a fluorescent signal. The test contains a second target, RNase P, a housekeeping gene. RNase P is detected in all the test specimens by the same mechanism, via a specific probe, labeled with reporter and quencher, which, during the extension phase of the PCR cycle, is degraded by the Taq DNA Polymerase causing the reporter dye to separate from the quencher dye and generate a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the ABI Fast. Components of PCR Master mix per reaction and cycling conditions are listed below.

INSTRUMENTS USED WITH TEST

The Triplex CII-SARS-CoV-2 rRT-PCR test is to be used with the bioMérieux easyMAG automated extraction platform using the NucliSENS easyMAG extraction kit or the QIAcube automated extraction system and the Applied Biosystem 7500 Fast Dx Real-Time PCR Instrument (ABI Fast) with software version 2.3.

REAGENTS AND MATERIALS

Components Included with the test (500 tests)

Reagent	**Manufacturer**	**Catalog #**
NA UltraSens One-Step Quantitative RT-PCR System	ThermoFisher	11732927
SARS-CoV-2 forward primer for N1 target	LGC Biosearch Technologies	Custom
SARS-CoV-2 reverse primer for N1 target	LGC Biosearch Technologies	Custom
SARS-CoV-2 FAM Probe for N1 target	LGC Biosearch Technologies	Custom
SARS-CoV-2 forward primer for N2 target	LGC Biosearch Technologies	Custom
SARS-CoV-2 reverse primer for N2 target	LGC Biosearch Technologies	Custom
SARS-CoV-2 Quasar 670 Probe for N2 target	LGC Biosearch Technologies	Custom
RNAse P forward primer	LGC Biosearch Technologies	Custom

RNAse P reverse primer	LGC Biosearch Technologies	Custom
RNAse P CAL Fluor Orange 560 probe	LGC Biosearch Technologies	Custom
SARS-CoV-2 N1 target Positive Control*	In-house	NA
SARS-CoV-2 N2 target Positive Control*	In-house	NA
Human specimen extraction control	ATCC	CRM-CCL-2
Extracted nucleic acid from HSC	In-house	NA

* The PC-1 (790 nt) and PC-2 (657 nt) were commercially synthesized as DNA and cloned into pUC57 plasmid DNA Vector (GenScript, USA). Plasmids were double digested using restriction enzymes to release target segments (New England Biolabs, USA). Gel purified target inserts were quantified by NanoDrop (Thermo Scientific) and used as templates for in vitro transcription with RiboMAX Large Scale RNA Production Systems (Promega, USA). In vitro transcribed RNA was DNase-treated, purified with Trizol (Thermo Fisher), DNase-treated again and quantitated by Agilent 4200 TapeStation. Based on optical density and insert size, RNA copy numbers were calculated and serially diluted (109 – 100 cp/µL) in distilled water with background of UltraPure™ Salmon Sperm DNA (50 ng/uL) (Thermofisher, USA).

Components Required but Not Included with the Test

Item	**Manufacturer**	**Catalog #**
BioMérieux NucliSENS® easyMag extraction system	bioMérieux	280140
Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument	Applied Biosystems	4406985
Applied Biosystems Optical Reaction Plate	ThermoFisher	4346906
MicroAmp™ Optical Adhesive Film	ThermoFisher	4360954
NucliSENS easyMag Magnetic Silica	bioMérieux	280133
NucliSENS easyMag Buffer 1	bioMérieux	280130
NucliSENS easyMag Buffer 2	bioMérieux	280131
NucliSENS easyMag Buffer 3	bioMérieux	280132
NucliSENS easyMag Lysis Buffer	bioMérieux	280134
NucliSENS easyMag Consumables	bioMérieux	280135
QIAcube HT extraction machine	QIAGEN	9001793
QIAamp 96 Virus QIAcube HT Kit	QIAGEN	57731
QIAcube HT Plasticware	QIAGEN	950067
Reagent Trough (with lid), 70 ml	QIAGEN	990554
Reagent Trough (with lid), 170 ml	QIAGEN	990556
Molecular Grade Ethanol		
Molecular Grade Isopropanol		
Microcentrifuge		
Vortex Mixer		

CONTROLS TO BE USED WITH THE TRIPLEX CII-SARS-CoV-2 rRT-PCR TEST

a) A no template control (NTC) is needed to eliminate 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 control (PC1) for the N1 target is needed to verify that the assay run is performing as intended and is added to RT-PCR master mix at 1.4 cp/µL. The PC1 is *in vitro* transcribed RNA of 790 nt, coding for the SARS-CoV-2 N1 target.

c) A positive control (PC2) for the N2 target is needed to verify that the assay run is performing as intended and is added to RT-PCR master mix at 1.4 cp/µL. The PC2is *in vitro* transcribed RNA of 657 nt, coding for the SARS-CoV-2 N2 target.

d) 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. This serves as the extraction positive control to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

e) A human specimen control (HSC) consists of human cell culture preparation from Hela cells known to contain RNase P template but negative for the SARS-CoV-2 targets. It serves both as an extraction control to validate extraction reagents and successful RNA extraction.

f) An extracted human specimen control (eHSC) consists of nucleic acid, extracted from the HSC. Its serves as a positive control in rRT-PCR for detection of human RNase P mRNA and as a negative control for detection of SARS-CoV-2 RNA.

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.

Triplex CII-SARS-CoV-2 rRT-PCR test Controls – Positive, Negative, and Internal:

NTC – negative for all targets detected (Ct ≥ 39)
PC1 – positive for SARS-CoV-2 N1 target (Ct < 35)
PC2 – positive for SARS-CoV-2 N2 target (Ct < 35)
RP – positive for RNase P (RP) target (Ct < 39)
HSC – negative for SARS-CoV-2 targets (Ct ≥ 39), positive for RNase P (RP) target (Ct < 39)
eHSC – negative for SARS-CoV-2 targets (Ct ≥ 39), positive for RNase P (RP) target (Ct < 39)

If any control does not perform as described above, the run is considered inconclusive and all specimens are repeated from extraction step.

Examination and Interpretation of Patient Specimen Results:

Note on RP internal control: all clinical samples should yield positive results for RP target at < 39 Ct. Samples that fail to show detection of RP within this range and SARS-CoV-2 N1 and N2 targets should be repeated from extraction step. If sample detects the SARS-CoV-2 N1 and N2 targets, the lack of amplification of RP target can still be valid.

SARS-CoV-2 Target (N1 and N2):

- NEGATIVE: All controls performed as expected; patient specimen does not detect SARS-CoV-2 N1 and N2 targets and RP target detected < 39 Ct. Report results to provider.
- POSITIVE: All controls performed as expected; patient specimen detects SARS-CoV-2 N1 and N2 targets or N1 target only and RP target detected < 39 Ct or RP target not detected. Report results to provider and appropriate health authorities.
- PRESUMPTIVE POSITIVE: All controls performed as expected; patient specimen detects SARS-CoV-2 N2 target only and RP target detected < 39 Ct or RP target not detected. Report results to provider and appropriate health authorities.
- INVALID: All controls performed as expected; SARS-CoV-2 N1and N2 target and RP target not detected. 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.
- EQUIVOCAL: All controls performed as expected; patient specimen detects SARS-CoV-2 N1 and/or N2 target(s) detected ≥ 39 Ct and RP target detected < 39 Ct or RP target not detected. Report results to provider and appropriate health authorities and recommend repeat sample collection. Report results only if RP is positive. If RP is negative, and N1/N2 Ct >39, the sample is re-extracted.

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 Triplex CII-SARS-CoV-2 rRT-PCR test at least 95% of the time. The LoD study was performed on the ABI Fast instrument. The preliminary LoD was established by testing nine different dilutions of T7 RNA *in vitro* transcripts[1] for SARS-COV-2 N1 and N2 targets that were serially 3-fold diluted with a background of salmon sperm DNA (1 ng/µL) in 50 ul Water. 50 µL of diluted T7 RNA transcripts were spiked in 200 µL of VTM with an OP swab from a healthy control. Total 250 µL was then mixed with 750 µL EasyMAG lysis buffer 1 and extracted according to manufacturer's instructions. The preliminary LoD was determined to be 0.28 cp/µL (3/3) (Table 1).

[1] Analytical and Clinical Studies were initially performed before genomic viral genomic RNA was widely available. A near-LoD study was performed to demonstrate the equivalency of *in vitro* transcribed RNA and viral genomic RNA. These data are reported under the clinical studies section.

The preliminary LoD was confirmed by testing 20 replicates of T7 RNA *in vitro* transcripts spiked into 200 ul of VTM with an OP swab from a healthy control at 0.28 cp/µL. The LoD was confirmed to be 0.28 cp/µL for the Triplex CII-SARS-CoV-2 rRT-PCR test.

Table 1. Results of the Preliminary LoD Experiment

	N1 target			N2 target		
Concentration (cp/µL)	Positive replicates	mean Ct value	Standard Deviation	Positive replicates	mean Ct value	Standard Deviation
200	1/1	24.7	NA	1/1	24.6	NA
66.7	1/1	26.6	NA	1/1	26.9	NA
22.2	1/1	28.2	NA	1/1	28.4	NA
7.4	1/1	29.9	NA	1/1	30.2	NA
2.48	3/3	30.5	0.6	3/3	30.7	0.8
0.84	3/3	32.8	1.3	3/3	32.7	0.8
0.28	3/3	33.9	0.9	3/3	34.7	0.6
0.1	0/3	NA	NA	0/3	NA	NA
0.04	0/1	NA	NA	0/1	NA	NA

NA = Not Available

Table 2. Results of the Analytical Sensitivity for the Triplex CII-SARS-CoV-2 rRT-PCR Test

	N1 target			N2 target		
Concentration (cp/µL)	Positive replicates	mean Ct value	Standard Deviation	Positive replicates	mean Ct value	Standard Deviation
0.28	19/20	34.8	1.6	20/20	35.1	0.9

Inclusivity

All available full-length SARS-CoV-2 genomic sequences were downloaded from GISAID on April 3 2020 (3210 sequences) and aligned to primer and probe sequences for N1 and N2 targets. Any genomic sequences containing ambiguous bases within primer or probe binding regions were excluded. 3153/3210 (98.22%) of SARS-CoV2 genomic sequences showed 100% nucleotide identity with N1 and N2 primers/probes. Fifty-six SARS-CoV-2 genomic sequences were not 100% identical to primer or probe sequences. There were no genomic sequences that showed greater than one nucleotide mismatch with any individual N1 or N2 primers and probes. One genomic sequence (hCoV19/Iceland/29/2020|EPI_ISL_417618) had a single mismatch in forward N1 primer and a single mismatch in reverse N2 primer. All other genomic sequences had at most one mismatch against one of the N1 or N2 primers and probes, ensuring that at least one complete primer/probe set was 100% identical.

2) Analytical Specificity:

The Triplex CII-SARS-CoV-2 rRT-PCR assay primer and probe sets was tested *in silico* for potential cross-reactivity with sequences of other representative respiratory viral and bacterial pathogens listed in Table 3. The default strict parameters of blastn analyses did

not identify potential homologous regions between the primers/probes and the target genome sequences due to high number of mismatches in very short oligonucleotide or probe sequences. To further exclude potential homology, we re-analyzed using relaxed blastn parameters , using a “word size” of 5, “match/mismatch scores” of 1 and -1, and increased the e-value cutoff to 1000 so that even very short alignments with weak matching scores could be identified. The N1 Forward and Reverse primers showed 91% and 89% homology, respectively, and the N1 probe showed 76% homology with SARS-CoV-1 (2003). Therefore, cross-reactivity of the N1 target with SARS-CoV-1 is not expected. The N2 Forward and Reverse primers showed 96% and 100% homology, respectively, and the N2 probe showed 85% homology with SARS-CoV-1 (2003). No other organism/viruses analyzed showed greater than 80% homology with any primers/probes from the Triplex CII-SARS-CoV-2 rRT-PCR assay.

Table 3. List of Viruses Analyzed *in silico* for Cross-Reactivity.

Name	Accession number	Name	Accession number	Name	Accession number
Human coronavirus 229E	NC_002645.1	Human rhinovirus 14	NC_001490.1	Snake adenovirus	NC_009989.1
Human coronavirus OC43	NC_006213.1	Human enterovirus A	NC_001612.1	Human adenovirus D	NC_010956.1
Human coronavirus HKU1	NC_006577.2	Human rhinovirus 89	NC_001617.1	Rhizobium phage 16-3	NC_011103.1
Human coronavirus NL63	NC_005831.2	Bovine enterovirus	NC_001859.1	Human adenovirus B2	NC_011202.1
SARS-coronavirus	NC_004718.3	Poliovirus	NC_002058.3	Human adenovirus B1	NC_011203.1
MERS-coronavirus	NC_019843.3	Porcine teschovirus 1	NC_003985.1	Murine adenovirus 3	NC_012584.1
Human Metapneumovirus (hMPV)	NC_039199.1	Porcine sapelovirus 1	NC_003987.1	Cercopithecine herpesvirus 5 strain 2715	NC_012783.2
Parainfluenza virus 1	NC_003461.1	Simian enterovirus A	NC_003988.1	Human adenovirus 54	NC_012959.1
Parainfluenza virus 2	NC_003443.1	Porcine enterovirus 9 strain UKG/410/73 polyprotein gene,	NC_004441.1	Turkey adenovirus 1	NC_014564.2
Parainfluenza virus 3	NC_001796.2	Avian sapelovirus	NC_006553.1	Murine adenovirus 2	NC_014899.1

Name	Accession number
Parainfluenza virus 4	NC_021928.1
Influenza A virus (A/New York/392/2004(H3N2)	NC_007366.1
	NC_007367.1
	NC_007368.1
	NC_007369.1
	NC_007370.1
	NC_007371.1
	NC_007372.1
	NC_007373.1
Influenza A virus (A/Korea/426/1968(H2N2))	NC_007375.1
	NC_007376.1
	NC_007377.1
	NC_007378.1
Possum enterovirus W1	NC_008714.1
Human rhinovirus C	NC_009996.1
Enterovirus J strain 1631	NC_010415.1
Enterovirus J strain N203	NC_013695.1
Enterovirus F strain BEV-261 polyprotein gene, complete	NC_021220.1
Enterovirus sp. isolate CPML_8109/08	NC_024073.1
Yak enterovirus strain SWUN-AB001	NC_029854.1
Enterovirus SEV-gx	NC_029905.1
Human enterovirus strain V13-0285, partial genome	NC_030454.1
Ovine adenovirus A	AC_000001.1
Bovine adenovirus B	AC_000002.1
Canine adenovirus 1	AC_000003.1
Duck adenovirus A	AC_000004.1
Fowl adenovirus E	NC_014969.1
Japanese eel endothelial cells-infecting virus	NC_015123.1
Simian adenovirus 49	NC_015225.1
Fowl adenovirus C	NC_015323.1
Raptor adenovirus A	NC_015455.1
Bat adenovirus 2	NC_015932.1
South polar skua adenovirus-1	NC_016437.1
Bat adenovirus TJM	NC_016895.1
Chimpanzee adenovirus Y25	NC_017825.1
Goose adenovirus 4	NC_017979.1
Bovine adenovirus 6 strain 671130	NC_020074.1
Simian adenovirus 20 strain ATCC VR-541	NC_020485.1
Titi monkey adenovirus ECC-2011	NC_020487.1

Name	Accession number
	NC_007379.1
	NC_007380.1
	NC_007381.1
	NC_007382.1
	NC_007383.1
Influenza A virus (A/Puerto Rico/8/1934(H1N1))	NC_002016.1
	NC_002017.1
	NC_002018.1
	NC_002019.1
	NC_002020.1
	NC_002021.1
	NC_002022.1
	NC_002023.1
Influenza B virus (B/Lee/1940)	NC_002204.1

Name	Accession number
Human mastadenovirus A	AC_000005.1
Human adenovirus D	AC_000006.1
Human adenovirus 2	AC_000007.1
Human adenovirus 5	AC_000008.1
Porcine adenovirus C	AC_000009.1
Simian adenovirus 21	AC_000010.1
Simian adenovirus 25	AC_000011.1
Murine adenovirus A	AC_000012.1
Fowl adenovirus D	AC_000013.1
Fowl adenovirus A	AC_000014.1
Turkey adenovirus A	AC_000016.1
Human adenovirus type 1	AC_000017.1
Human adenovirus type 7	AC_000018.1
Human adenovirus	AC_000019.1

Name	Accession number
Simian adenovirus C isolate BaAdV-2	NC_021168.1
Fowl adenovirus 5 strain 340	NC_021221.1
Pandoravirus dulcis	NC_021858.1
Simian adenovirus 18	NC_022266.1
Turkey adenovirus 4 isolate TNI1	NC_022612.1
Turkey adenovirus 5 isolate 1277BT	NC_022613.1
Ralstonia phage RSK1 DNA	NC_022915.1
California sea lion adenovirus 1 strain Zc11-030	NC_024150.1
Pigeon adenovirus 1 complete genome, strain IDA4	NC_024474.1
Duck adenovirus 2 strain GR	NC_024486.1
Lizard adenovirus 2 isolate 23-06	NC_024684.1
Simian adenovirus DM-2014 isolate 23336	NC_025678.1
Psittacine adenovirus 3 isolate HKU/Parrot 19	NC_025962.1
Equine adenovirus	NC_027705.1

Name	Accession number
	NC_002205.1
	NC_002206.1
	NC_002207.1
	NC_002208.1
	NC_002209.1
	NC_002210.1
	NC_002211.1
Respiratory syncytial virus	NC_001803.1
Chlamydia pneumoniae	NC_005043.1
Haemophilus influenzae	NZ_LN831035
Legionella pneumophila	NZ_LR134380.1
Mycobacterium tuberculosis	NC_000962.3

Name	Accession number
type 35	
Canine adenovirus type 2	AC_000020.1
Porcine adenovirus 3	AC_000189.1
Tree shrew adenovirus 1	AC_000190.1
Bovine adenovirus A	AC_000191.1
Fowl adenovirus D	NC_000899.1
Murine adenovirus A	NC_000942.1
Human adenovirus C	NC_001405.1
Human adenovirus F	NC_001454.1
Human adenovirus A	NC_001460.1
Fowl adenovirus A	NC_001720.1
Canine adenovirus	NC_001734.1
Duck adenovirus A	NC_001813.1

Name	Accession number
2 isolate EAdV2.385/75.9	
Skunk adenovirus PB1	NC_027708.1
Simian adenovirus 13 strain P-9	NC_028103.1
Simian adenovirus 16 strain C-8	NC_028105.1
Simian adenovirus 19 strain AA153	NC_028107.1
Simian adenovirus 8 strain P-5	NC_028113.1
Unidentified adenovirus isolate CSPAdV_2	NC_030116.1
Tokyovirus A1 DNA, nearly complete genome	NC_030230.1
Equine adenovirus 1 strain M1	NC_030792.1
Pigeon adenovirus 2 isolate YPDS-Y-V1.A19.11-2013	NC_031503.1

Name	Accession number
Streptococcus pneumoniae	NZ_LN831051.1
Streptococcus pyogenes	NZ_LN831034
Bordetella pertussis	NC_018518.1
Mycoplasma pneumoniae	NZ_CP010546.1
Pneumocystis jirovecii (PJP)	MK984200
Candida albicans	NC_018046
Pseudomonas aeruginosa	NC_002516.2
Staphylococcus epidermidis ASM609437v1	NZ_CP035288.1
	NZ_CP035289.1
	NZ_CP035290.1
Streptococcus salivarius	NZ_LR134274.1
Human enterovirus D	NC_001430.1
Human enterovirus B	NC_001472.1

Name	Accession number
Bovine adenovirus 3 complete genome	NC_001876.1
Hemorrhagic enteritis virus	NC_001958.1
Frog adenovirus 1	NC_002501.1
Bovine adenovirus type 2	NC_002513.1
Bovine adenovirus D	NC_002685.2
Porcine adenovirus 5	NC_002702.1
Human adenovirus E	NC_003266.2
Garlic virus A	NC_003375.1
Ovine adenovirus 7	NC_004037.2
Bovine adeno-associated virus	NC_005889.1
Simian adenovirus 3	NC_006144.1
Macacine herpesvirus 3	NC_006150.1
Simian adenovirus 1	NC_006879.1

Name	Accession number

Cross-reactivity of the Triplex CII-SARS-CoV-2 rRT-PCR assay was also evaluated using extracted nucleic acid from a panel of organisms listed in Table 4. Extracted nucleic acids were spiked in to 250 µL of VTM collected with oropharyngeal swabs samples from a healthy individual, extracted using EasyMag platform, and measured on the ABI Fast. There was no cross-reactivity observed for any of the tested organisms/viruses.

Table 4. Pathogens Tested for Cross Reactivity Against the N1 and N2 Primers/Probe Set.

Name	Source	copies / reaction	Triplex CII-SARS-CoV-2 rRT-PCR assay result
Haemophilus influenzae	ATCC 10211	NA*	ND
Mycobacterium tuberculosis	Clinical specimen	2.10E+04	ND
Streptococcus pneumoniae	NR-20805	2.80E+05	ND
Streptococcus pyogenes	NR-50136	NA*	ND
Bordetella pertussis	NR-42457	4.32E+05	ND
Candida albicans	ATCC MYA2876	4.97E+04	ND
Pseudomonas aeruginosa	NR-50573	NA*	ND
Human CoV 229E	ATCC VR-740	~1.00E+08	ND
Human CoV OC43	ATCC VR-1558	~1.00E+05	ND
Human CoV HKU1	ATCC VR-3262SD	~1.00E+05	ND
Human CoV NL63	ATCC VR-3263SD	~1.00E+05	ND
Human adenovirus 3	ATCC VR-847D	4.60E+04	ND
Human Metapneumovirus (hMPV)	clinical isolate	3.30E+03	ND
Parainfluenza virus - 1	ATCC VR-94	4.88E+03	ND
Parainfluenza virus - 2	ATCC VR-92D	2.89E+04	ND
Parainfluenza virus - 3	ATCC VR-1782	1.29E+05	ND
Influenza A Strain A/Panama/2007/99	Stored virus culture	1.20E+04	ND
Inflenza B/Malaysia/2506/2004	Stored virus culture	7.50E+05	ND
Enterovirus (EVD68)	VR-1076 (ATCC)	4.45E+05	ND
Respiratory syncytial virus (RSV)	VR-1540 (ATCC)	1.95E+05	ND
Rhinovirus	HRV16 (ATCC)	5.00E+05	ND
Enterovirus (EVA71)	Tainan -4643 (BEI)	4.90E+05	ND
Pooled human nasal wash	NA	NA	ND

NA = Not Available
ND = Not Detected
* concentrations were not available from ATTC. 50 µL of the stock provided by ATCC were spiked directly into 200 µL OP collected in VTM.

3) Clinical Evaluation

Testing with Contrived Samples

A contrived clinical study was conducted to evaluate the performance of the Triplex CII-SARS-CoV-2 rRT-PCR test. A total of 60 individual oropharyngeal clinical specimens, collected in VTM and presumed negative for SARS-CoV-2, were used in this study.

Positive samples were prepared by spiking T7 RNA *in vitro* transcript[2] for SARS-COV-2 N1 and N2 targets into OP matrix at 2X, 27X, 81X, 243X and 729X LoD. Samples were extracted and tested, blinded to the analyst, using the EasyMag nucleic acid extraction platform and ABI Fast instrument. The positive and negative percent agreements between the Triplex CII-SARS-CoV-2 rRT-PCR test and the expected results are shown below.

Table 5. Performance of the Triplex CII-SARS-CoV-2 rRT-PCR Test on ABI Fast with Contrived Samples:

SARS-CoV-2 transcript concentration	Number of OP Swabs	N1 target		N2 target	
		Mean Ct Value	Percent Agreement (95% CIs)	Mean Ct Value	Percent Agreement (95% CIs)
2X LoD	20	32.8 ± 0.7	95% (76.4 - 99.1)[a]	33.0 ± 0.8	90% (69.9 - 97.2)[a]
27X LoD	2	30.1 ± 0.5	100% (34.2- 100)	30.4 ± 0.3	100% (34.2- 100)
81X LoD	2	28.7 ± 0.3	100% (34.2- 100)	28.3 ± 0.1	100% (34.2- 100)
243X LoD	3	26.9 ± 0.3	100% (43.9 - 100)	27.3 ± 0.3	100% (43.9 - 100)
729X LoD	3	25.3 ± 0.2	100% (43.9 - 100)	24.9 ± 0.3	100% (43.9 - 100)
Negative	30	NA	100% (88.6 - 100)	NA	100% (88.6 - 100)

NA = Not Available

[a]Sample number 17 failed detection of both the N1 and N2 targets and sample number 2 failed detection of the N2 target only, therefore, the overall result at 2X LoD is 95% agreement with expected results.

Performance of the Triplex CII-SARS-CoV-2 rRT-PCR test on ABI Fast against the expected results are:

Positive Percent Agreement 29/30 = 96.7% (95% CI: 83.3% - 99.4%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)

Equivalency of RNA transcripts and Viral Genomic RNA

In order to show equivalency of *in vitro* transcribed RNA with viral genomic RNA, a study was performed to demonstrate the Triplex CII-SARS-CoV-2 rRT-PCR test on ABI Fast can detect viral genomic RNA at near-LoD, determined using *in vitro* transcribed RNA. Full length SARS-CoV-2 viral genomic RNA, sourced from infected Vero E6 cells (2019-nCoV/USA-WA1/2020; accession MN985325 grown in Vero E6 Cat# ATCC CRL-158), was extracted in a BSL3 lab using the Easymag platform. Twenty individual oropharyngeal swab samples, collected in VTM, were spiked with viral genomic RNA at 1.75X LoD. Spiked samples were extracted on the EasyMag platform and measured on the ABI Fast. The results, summarized in Table 6, support the claim that the Triplex CII-SARS-CoV-2 rRT-PCR test is able to detect viral genomic RNA, near the LoD, with the same accuracy (similar Ct values) as *in vitro* transcribed RNA.

[2]Analytical and Clinical Studies were initially performed before genomic viral genomic RNA was widely available. A bridging study was performed to demonstrate the equivalency of *in vitro* transcribed RNA and viral genomic RNA. These data are reported under the clinical studies section.

Table 6. Performance of the Triplex CII-SARS-CoV-2 rRT-PCR Test Using Contrived Samples Prepared with Viral Genomic RNA:

SARS-CoV-2 RNA	Number of OP Swabs	N1 target		N2 target		RP Target	
		Mean Ct Value	Percent Agreement (95% CIs)	Mean Ct Value	Percent Agreement (95% CIs)	Mean Ct Value	Percent Agreement (95% CIs)
1.75X LoD	20	32.9 ± 0.3	100% (83.9 - 100)	34.4 ± 1.0	100% (83.9 - 100)	31.1 ± 2.0	100% (83.9 - 100)

Testing with Clinical Specimens

Five positive and five negative patient samples (Patient 1 – 10) were received from Columbia University Pathology & Cell Biology laboratory, tested using the "New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel" (EUA200002). Samples were tested in duplicate using the Triplex CII-SARS-CoV-2 rRT-PCR assay. All results were concordant and fulfill the requirement for confirmatory testing for at least five positive and five negative specimens.

The sponsor submitted an additional 30 samples (Patient 11 – 40), again received from Columbia University Pathology & Cell Biology laboratory, tested using the "New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel" (EUA200002). Samples were tested in duplicate using the Triplex CII-SARS-CoV-2 rRT-PCR assay. All results were concordant and fulfill the requirement for evaluation of clinical specimens.

Results from both sets of patient samples were pooled to generate the performance data summarized in Table 7 below.

Table 7. Results of Clinical Specimens Tested with the Triplex CII SARS-CoV-2 RT-PCR Assay and with an Authorized Test

Sample ID	Triplex CII-SARS-CoV-2 rRT-PCR assay							New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel Results
	Ct values 1			Ct values 2			Results	
	N1	N2	RP	N1	N2	RP		
Patient 1	17.2	17.3	40.9	17.5	17.6	40.3	POSITIVE	POSITIVE
Patient 2	17.7	17.8	40.3	17.8	18	39.3	POSITIVE	POSITIVE
Patient 3	29.8	30.3	27.3	31	31.2	27.4	POSITIVE	POSITIVE
Patient 4	21.1	21.3	24.4	21.1	21.3	24.3	POSITIVE	POSITIVE
Patient 5	24.2	24.4	26.2	23.9	24.3	26.2	POSITIVE	POSITIVE
Patient 6	ND	ND	29.5	ND	ND	29.7	NEGATIVE	NEGATIVE
Patient 7	ND	ND	30.3	ND	ND	30.2	NEGATIVE	NEGATIVE
Patient 8	ND	ND	28.8	ND	ND	28.7	NEGATIVE	NEGATIVE
Patient 9	ND	ND	28.6	ND	ND	28.7	NEGATIVE	NEGATIVE
Patient 10	ND	ND	27.2	ND	ND	27.2	NEGATIVE	NEGATIVE
Patient 11	17.2	17.3	40.9	17.5	17.6	40.3	POSITIVE	POSITIVE
Patient 12	17.7	17.8	40.3	17.8	18	39.3	POSITIVE	POSITIVE
Patient 13	29.8	30.3	27.3	31	31.2	27.4	POSITIVE	POSITIVE
Patient 14	21.1	21.3	24.4	21.1	21.3	24.3	POSITIVE	POSITIVE
Patient 15	24.2	24.4	26.2	23.9	24.3	26.2	POSITIVE	POSITIVE
Patient 16	ND	ND	29.5	ND	ND	29.7	NEGATIVE	NEGATIVE
Patient 17	ND	ND	30.3	ND	ND	30.2	NEGATIVE	NEGATIVE
Patient 18	ND	ND	28.8	ND	ND	28.7	NEGATIVE	NEGATIVE
Patient 19	ND	ND	28.6	ND	ND	28.7	NEGATIVE	NEGATIVE
Patient 20	ND	ND	27.2	ND	ND	27.2	NEGATIVE	NEGATIVE
Patient 21	34.6	34.3	30.6	36.5	34.7	30.1	POSITIVE	POSITIVE
Patient 22	20.9	21.9	27.6	21.0	21.9	26.9	POSITIVE	POSITIVE
Patient 23	ND	ND	25.8	ND	ND	25.8	NEGATIVE	NEGATIVE
Patient 24	ND	ND	25.5	ND	ND	25.6	NEGATIVE	NEGATIVE
Patient 25	ND	ND	29.8	ND	ND	29.8	NEGATIVE	NEGATIVE

Sample ID	Triplex CII-SARS-CoV-2 rRT-PCR assay							New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel Results
	Ct values 1			Ct values 2			Results	
	N1	N2	RP	N1	N2	RP		
Patient 26	42.9	ND	28.6	ND	ND	28.1	NEGATIVE	NEGATIVE
Patient 27	32.3	33.3	31.0	31.6	32.9	30.8	POSITIVE	POSITIVE
Patient 28	ND	ND	28.2	ND	ND	28.3	NEGATIVE	NEGATIVE
Patient 29	ND	ND	28.0	ND	ND	28.0	NEGATIVE	NEGATIVE
Patient 30	ND	ND	28.6	ND	ND	28.7	NEGATIVE	NEGATIVE
Patient 31	ND	ND	25.1	ND	ND	25.0	NEGATIVE	NEGATIVE
Patient 32	ND	ND	27.6	ND	ND	27.5	NEGATIVE	NEGATIVE
Patient 33	ND	ND	26.1	ND	ND	26.4	NEGATIVE	NEGATIVE
Patient 34	27.9	28.7	27.8	27.8	28.9	27.7	POSITIVE	POSITIVE
Patient 35	ND	ND	29.8	ND	ND	29.6	NEGATIVE	NEGATIVE
Patient 36	ND	ND	27.5	ND	ND	27.5	NEGATIVE	NEGATIVE
Patient 37	15.6	16.1	41.1	15.6	16.2	38.9	POSITIVE	POSITIVE
Patient 38	21.1	22.2	34.7	20.9	22.3	36.9	POSITIVE	POSITIVE
Patient 39	29.9	30.8	32.5	29.4	30.6	32.3	POSITIVE	POSITIVE
Patient 40	39.8	ND	28.9	ND	ND	28.8	NEGATIVE	NEGATIVE

Performance of the Triplex CII-SARS-CoV-2 rRT-PCR test against the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel is:
Positive Percent Agreement 17/17 = 100% (95% CI: 81.6% - 100%)
Negative Percent Agreement 23/23 = 100% (95% CI: 85.7% - 100%)

4) Validation of Additional Extraction Platforms

Validation of the QIAcube Extraction Platform

On August 3, 2020, bridging data, comparing the QIAcube extraction method to the bioMérieux easyMAG extraction method, was submitted in support of adding the QIAcube extraction method to the list of extraction methods validated with the Triplex CII-SARS-CoV-2 rRT-PCR test. Contrived samples were prepared by diluting SARS-CoV-2 viral RNA (Cat# ATCC CRL-158) into pooled oropharyngeal swab matrix in VTM. A series of dilutions was prepared and tested, using each extraction platform in parallel, in triplicate. The LoD was defined as the lowest concentration at which all

three replicates returned a positive result (Ct < 39). The results, summarized in Table 8, showed an LoD of 2.8 x 10^2 cp/mL for both methods. These results support the addition of the QIAcube extraction platform to the Triplex CII-SARS-CoV-2 rRT-PCR test.

Table 8. Comparative LoD for the QIAcube and bioMérieux easyMAG extraction platforms on the Triplex CII-SARS-CoV-2 rRT-PCR test

		QIACube Extraction system						bioMérieux easyMAG					
		N1 target			N2 target			N1 target			N2 target		
Panel member	Analyte concentration (cp/mL)	positives (n/3)	mean Ct	Ct Stdev	positives (n/3)	mean Ct	Ct Stdev	positives (n/3)	mean Ct	Ct Stdev	positives (n/3)	mean Ct	Ct Stdev
1	6.67 x 10^4	3/3	27.68	0.05	3/3	28.11	0.10	3/3	28.71	0.15	3/3	30.05	0.02
2	2.22 x 10^4	3/3	29.16	0.08	3/3	29.76	0.06	3/3	30.00	0.12	3/3	31.08	0.13
3	7.40 x 10^3	3/3	30.58	0.03	3/3	31.32	0.18	3/3	31.58	0.18	3/3	32.37	0.16
4	2.48 x 10^3	3/3	32.41	0.09	3/3	33.11	0.13	3/3	31.82	0.13	3/3	32.72	0.21
5	8.40 x 10^2	3/3	33.83	0.13	3/3	34.61	0.20	3/3	35.39	0.50	3/3	35.69	0.49
6	2.80 x 10^2	3/3	35.39	0.27	3/3	36.55	0.22	3/3	36.04	0.10	3/3	36.86	0.97
7	1.00 x 10^2	2/3	38.01	0.88	2/3	38.99	1.67	2/3	38.49	3.28	0/3	NA	NA
8	4.00 x 10^1	0/3	39.94	0.31	0/3	40.58	0.07	0/3	NA	NA	0/3	NA	NA

NA = Not Applicable. Results did not return any Ct values.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Columbia University Laboratory of Personalized Genomic Medicine;
- 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 Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.