ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY THE UNIVERSITY OF NEBRASKA MEDICAL CENTER (UNMC) NECOV19 RT-PCR ASSAY

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

(The NEcov19 RT-PCR Assay will be performed at the Nebraska Medicine Clinical Laboratory certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions for Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The NEcov19 RT-PCR Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in human nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate swabs, nasal washes, nasal aspirates and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Nebraska Medicine Clinical Laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. 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 management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The NEcov19 RT-PCR Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization (EUA).

DEVICE DESCRIPTION AND TEST PRINCIPLE

The NEcov19 RT-PCR Assay is a one-step real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. The SARS-CoV-2 primer and probe set is designed to detect RNA from SARS-CoV-2 in respiratory specimens from individuals suspected of COVID-19 by their healthcare provider.

The assay detects a nucleic acid sequence from the SARS-CoV-2 E gene. The human RNase P RNA is also detected as an internal control. The RNase P internal control serves to confirm specimen cellularity, adequate extraction of nucleic acids, and intact reverse transcription and PCR amplification. The SARS-CoV-2 E-gene assay and the RNase P assay are performed in two separate wells.

Total nucleic acids are extracted from viral transport medium into which an upper respiratory tract swab specimen (such as a nasopharyngeal swab) has been placed.

Nucleic Acids Extraction is accomplished using either one of the following methods:

- Automated extraction using the Roche MagNA Pure Total Nucleic Acid Kit with External Lysis Buffer (Roche Applied Science, Indianapolis, IN), Catalog #03038505001 on the Roche MagNA Pure Compact instrument.
- Automated extraction using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit on the ThermoFisher KingFisher Flex platform.
- Manual extraction using the QiaAmp DSP Viral RNA Mini Kit

RNA is reverse-transcribed to form cDNA, which is then amplified by PCR using primers to each of the two gene targets. The PCR products are detected with probes designed to anneal to the specific target sequence. The probes are tagged with FAM (fluorescein) dye which fluoresces when probes hybridize to target DNA sequence and are released by 5' nuclease activity. The RT-PCR assays are performed on the Applied Biosystems QuantStudioDx RT-PCR instrument. The instrument measures the fluorescence in each well at the completion of each PCR cycle, generating amplification curves. The determination of "detected" is based on the number of cycles required for the amplification curve to pass a threshold.

INSTRUMENTS USED WITH TEST

The NEcov19 RT-PCR Assay is to be used with the following instrumentation:

- Roche MagNA Pure Compact
- ThermoFisher KingFisher Flex platform
- QuantStudio Dx with QuantStudio Test Development Software version 1.0.3

REAGENTS AND SUPPLIES

Reagents

1) Invitrogen Superscript III Platinum One-Step Quantitative RT-PCR Kit, Cat#11732-088.
2) Roche MagNA Pure Total Nucleic Acid Kit with External Lysis Buffer (Roche Applied Science, Indianapolis, IN), Catalog #03038505001. Storage: Room Temperature.
3) MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (ThermoFisher, Cat# A48383)
4) QiaAmp DSP Viral RNA Mini Kit (Qiagen Cat#61904)
5) Nuclease Free Water (SRW)
6) Primers and probes for E and RNase P targets (IDT)
7) RNA Storage Solution (RSS) (Ambion, Inc. Cat#7001)

Supplies:

1) Solid-front or wrap-around gown
2) Lab coat
3) Gloves
4) Fit-tested N-95 respirator
5) Face shield, safety goggles, or eyeglasses
6) Bleach, 0.5-0.7% sodium hypochlorite solution
7) 70% ethanol
8) Sterile nuclease-free filtered pipette tips
9) RNase/DNase-free 1.5ml polypropylene microcentrifuge tubes
10) Applied Biosystems MicroAmp Fast 8-strip 0.1ml, Cat#4358293 or Applied Biosystems MicroAmp Optical 8-cap strip, Cat#4323032
11) Applied Biosystems MicroAmp Fast Optical 96-Well Reaction Plate with Barcode, 0.1µl, Part#4346906, Part#4346907 or Part#4366932
12) KingFisher Deepwell 96 Plate, Cat#95040460, or equivalent
13) KingFisher 96 KF Plate, Cat#97002540, or equivalent
14) KingFisher Flex 96 tip comb for DW magnets, Cat#97002534
15) Reagent Reservoir 175 ml basin, Cat#9317-0175 or equivalent
16) Sterile nuclease-free filtered pipette tips

CONTROLS TO BE USED WITH THE NECOV19 RT-PCR ASSAY

1) No Template Control (NTC)

   The NTC consists of sterile water. This control is needed to ensure that there is no contamination of the RT-PCR reagents during RT-PCR assay set-up. The NTC is included in each RT-PCR reaction run for each assay. The NTC should not amplify in any assay.

2) Positive Control (PC)

   The PC is comprised of a quantified EXACT Diagnostics (EDX) SARS-CoV-2 RNA Standard (200,000 copies/mL, Cat#COV019) diluted in EXACT Diagnostics (EDX) SARS-CoV-2 Negative (Cat#COV000). The working concentration of the PC is 40 copies/µL, approximately 5xLoD).

   Alternatively, synthetic single stranded DNA (IDT custom synthesis) spanning the target region of the E-gene target diluted in nuclease free water (working concentration of 10^3 copies/µL, approximately 10xLoD) can also be used as the PC.

   The PC is included in each RT-PCR reaction run. This PC is needed to ensure test reagents are properly detecting SARS-CoV-2. The PC should test positive for the SARS-CoV-2 E-gene assay.

3) Negative Extraction Control (NEC)

Negative extraction control (lysis buffer) controls for cross contamination or reagent contamination associated with extraction. It should be performed with every batch of extraction.

4) Internal control (IC) (RNase P)

The RNase P RNA from human cellular materials is endogenous of human respiratory samples, and therefore serves as the sample adequacy and extraction control. The IC also serves as control for detection of inhibitors of the RT-PCR process.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of clinical specimen results. If the controls are not valid, the clinical specimen results cannot be interpreted.

1) Interpretation of Controls

1. The positive control test is valid if the E-gene assay is detected at a Ct <35.
    a. Failure of the positive control may indicate a pipetting error, degradation of the control material, or failure of amplification due to reagent degradation, error in the preparation of reaction mix or prime/probe mix, or instrument error.
    b. If the E-gene assay amplifies at Ct ≥35 or fails to amplify, the positive control RT-PCR reaction should be repeated. If a second invalid result is obtained, a new preparation of positive control material should be prepared and tested.
    c. If the positive control Ct falls above the value established during assay validation (i.e., mean Ct + 2xStandard Deviation (SD)) for the PC, the PC data should be interpreted with caution and repeat testing should be carefully considered. Notify the pathologist for each occurrence and seek advice.
    d. If the positive control fails during a run of patient samples, negative patient samples should be repeated; positive patient samples do not require repeat testing.

2. Negative control tests (No Template Control and Negative Extraction Control) are valid if no amplification is observed at Ct <40. If a Ct <40 result is obtained, the amplification plot should be reviewed to differentiate between true exponential amplification (indicative of an invalid result) and non-exponential upward creep in background fluorescence (valid result).
    a. Failure of the No Template Control may indicate cross-contamination of the PCR plate or contamination of the water. If persistent amplification is observed upon repeat testing, replace the water and examine workflow practices that may be implicated in generating contamination.
    b. Failure of the Negative Extraction Control may indicate cross-contamination of the PCR plate or contamination during the extraction process. If persistent amplification is observed upon repeat testing, re-extract positive samples that were processed on the same run as the invalid Negative Extraction Control. Examine extraction workflow practices that may be implicated in generating contamination.

3. RNase P (Internal Control) tests are valid if detected at a Ct ≤30, indicating adequate cellularity of the specimen, adequate nucleic acid extraction, and lack of significant inhibitors in the specimen.
    a. Failure of the RNase P internal control may result from:
        - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity
        - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation
        - Improper assay set up
        - Reagent or equipment malfunction
        - RT-PCR inhibitors in the specimen

    b. If the RNaseP Ct is >30, and if the sample is negative for the E-gene target, re-extract the sample and repeat the test.

    c. If the RNaseP is still >30, consult the Medical Director.

2) Interpretation of Sample Results

1. A sample result is interpreted as **negative (NOT DETECTED)** for the presence of SARS-CoV-2 RNA if the amplification curve for the E-gene assay does **NOT** cross the threshold at Ct <40 and the positive control and RNase P internal control for the sample are valid.
2. A sample result is interpreted as **positive (DETECTED)** for the presence of SARS-CoV-2 RNA if the amplification curve for the E-gene assay crosses the threshold at Ct <40 and the NTC and NEC controls are valid.
    a. Amplification curves should be visually inspected to differentiate between true exponential amplification and non-exponential upward creep in background fluorescence. If it is not clear whether the fluorescence curve is indicative of exponential amplification or background creep the reaction should be repeated with E and RNaseP targets.
        - If the E-gene target amplifies at Ct<40 on repeat test, and all controls are valid, result is interpreted as **positive** for SARS-CoV-2 RNA (**DETECTED**).
        - If the E-gene target is **negative** on repeat test, the result is interpreted as **negative**. Consult the laboratory director for further action.
    b. An RNase P internal control failure does NOT invalidate the detection of SARS-CoV-2 RNA in a clinical specimen.
3. A sample result is interpreted as **INVALID** for the presence of SARS-COV-2 RNA if the amplification curve for the E-gene assay does not cross the threshold at Ct<40 **AND** the RNase P internal control for the sample is invalid (Ct>30).
    a. Re-extract the specimen for repeat testing. If the re-extracted sample is persistently invalid, the result is interpreted as invalid due to specimen insufficiency or the presence of PCR inhibitors in the specimen.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity

An LoD estimation study was conducted utilizing the Exact Diagnostics SARS-CoV-2 RNA standard, which contains quantitated synthetic RNA transcripts containing five SARS-CoV-2 gene targets (E, N, ORF1ab, RdRp, and S). The RNA standard was diluted in Ambion RNA storage solution to 5000, 2000, 1000, 500, 250, and 125 RNA copies/mL. 400µL of each RNA dilution was extracted in triplicate using the ThermoFisher KingFisher Flex platform with elution in 50 µL elution buffer. The results of the LoD estimation study are shown in Table 1 below. 1000 copies/mL was the lowest dilution with 3/3 positive results. 1000 copies/mL of sample input equates to 8 copies/µL of RNA template in the RT-PCR reaction assuming 100% extraction efficiency.

Table 1: LoD Estimation Study with Extraction of Quantitated RNA Standard

RNA standard concentration (copies/mL)	NEcov19 positive results (extraction replicates)	NEcov19 E-gene Ct values
5000	3/3	31.7, 30.7, 30.8
2000	3/3	33.0, 32.8, 32.7
1000	3/3	33.6, 33.2, 33.5
500	2/3	34.7, 35.4
250	2/3	36.4, 37.8
125	1/3	38.0

To confirm the estimated LoD, 20 extraction replicates of the SARS-CoV-2 RNA standard diluted to 1000 copies/mL using the ThermoFisher KingFisher Flex platform were tested. The results of the LoD confirmation study are shown in Table 2 below. 20/20 extraction replicates were positive at 1000 copies/mL.

Table 2: LoD Confirmation Study with Extraction of Quantitated RNA Standard using the KingFisher Extraction Method

RNA standard concentration (copies/mL)	NEcov19 positive results	NEcov19 E-gene Ct values		
1000	20/20	34.27	33.05	33.09
		33.17	33.09	33.37
		33.29	33.57	33.33
		32.94	33.80	33.86
		32.83	33.75	33.28
		33.59	32.85	33.99
		33.63	33.21	
		Mean Ct = 33.40; Std = 0.40		

To further confirm the performance of the NEcov19 assay around the established LoD in clinical specimen matrix, a dilution series of a known SARS-CoV-2-positive NP swab

specimen diluted in pooled SARS-CoV-2-negative NP swab in VTM specimens were tested. Extraction replicates using the ThermoFisher KingFisher Flex platform were performed at 10 dilution points from $1:10^2$ to $1:10^8$ dilutions. The $1:10^7$ dilution point was the lowest dilution with all positive replicates (Table 3 below). Applying a standard curve generated from extracted SARS-CoV-2 quantitated RNA standard, the $1:10^7$ dilution was estimated to contain approximately 924 copies/mL of SARS-CoV-2 RNA, consistent with the confirmed LoD. In total, 20/20 extraction replicates at 1-2x LoD generated positive results in this study.

Table 3: Assay Analytical Sensitivity Performance in Diluted Clinical NP Swab Specimen using the KingFisher Extraction Method

Dilution factor of SARS-CoV-2-postive NP swab in VTM specimen	NEcov19 positive results (extraction replicates)	Necov19 E gene Ct values (mean)
$1:10^2$	6/6 (100%)	17.63
$1:10^3$	6/6 (100%)	20.55
$1:10^4$	6/6 (100%)	23.92
$1:10^5$	6/6 (100%)	27.26
$1:10^6$	7/7 (100%)	30.67
$1:5x10^7$	3/3 (100%)	31.86
$1:1.25x10^7$	10/10 (100%)	33.94
$1:10^7$	10/10 (100%)	34.14
$1:5x10^8$	6/9 (66.7%)	36.29
$1:10^8$	3/8 (37.5%)	37.25

To further assess analytical sensitivity of the NEcov19 RT-PCR Assay relative to that of commercially available SARS-CoV-2 NAAT EUA tests, an additional comparative LoD study was performed testing a panel of serial dilutions from a SARS-CoV-2-positive NPS in VTM specimen. In this additional study, the dilutions were extracted using the Roche MagNA Pure Total Nucleic Acid Kit with External Lysis Buffer on the MagNA pure Compact extraction instrument (100 µL sample input volume with 50 µL elution volume) in triplicate and tested using the NEcov19 RT-PCR Assay. The same dilutions were also tested using the Roche cobas SARS-CoV-2 and the Abbott ID NOW COVID-19 tests per the appropriate Instructions for Use. The performance of the NEcov19 RT-PCR Assay was compared directly to that of the Roche cobas SARS-CoV-2 and the Abbott ID NOW COVID-19 tests that were both authorized to be used under an EUA. The study results are summarized in Table 4 below.

Table 4. Comparison of NEcov19 Assay Performance with Other EUA Tests using the MagNA Pure Extraction Method

Dilution	NEcov19 (100 µL input)						cobas SARS-CoV-2 (700 µL input)				ID NOW COVID19 (200 µL input)		
	E-gene			RNase P			E-gene target		Orf1a target		RdRp target		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep3
$1:10^2$	20.6	20.5	20.6	23.7	23.3	21.2	20.7	20.8	20.4	20.3	--	--	--
$1:10^3$	23.8	23.8	29.2	23.4	23.3	30.8	23.7	23.6	23.4	23.2	POS	--	--
$1:10^4$	27.3	27.2	26.9	23.4	23.4	23.7	27.3	27.4	26.7	26.8	POS	--	--
$1:10^5$	30.9	33.5	30.8	23.7	24.5	23.9	29.9	30.8	28.1	29.6	POS	POS	POS

$1:10^6$	33.8	34.0	34.1	23.7	24.0	23.8	33.5	33.2	31.5	31.3	NEG	POS	NEG
$1:10^7$	39.3	37.6	NEG	26.0	23.7	23.5	36.0	35.3	32.9	33.5	NEG	NEG	NEG
$1:10^8$	36.0	NEG	NEG	25.5	23.4	23.5	NEG	NEG	NEG	NEG	NEG	NEG	NEG

Based on the results from this limited comparative study, it appears that the analytical sensitivity of the NEcov19 RT-PCR Assay is comparable to that of the Roche cobas SARS-CoV-2 and superior than the Abbott ID NOW COVID19

2) *Inclusivity (analytical sensitivity)*

The inclusivity of the NEcov19 RT-PCR Assay was evaluated by *in silico* analysis. Biweekly review of current betacoronavirus databases for mutations in the E-gene target primer and probe sites using the WashU Epigenome Browser (epigenomegateway.wustl.edu/browser) was carried out. As of May 14, 2020, this review included 2062 genomes. There were no polymorphisms present within the regions targeted by the primers of the NEcov19 RT-PCR Assay. There were the following three genomes contain polymorphisms within the regions targeted by the probe of the NEcov19 RT-PCR Assay that may impact the reactivity of the NEcov19 RT-PCR Assay:

NCBI Accession	Country of Origin	Date Submitted	Mismatch	Location of Mismatch
MT039890.1	South Korea	2/1/2020	T to A	4th base pair from the 3' end of the probe
MT263389.1	Washington, USA	3/30/2020	T to G	4th base pair from the 3' end of the probe
MT350246.1	Virginia, USA	4/17/2020	C to T	7th base pair from the 3' end of the probe

3) *Cross-reactivity (Analytical Specificity)*

a) *Cross-reactivity*

i. in silico cross-reactivity

To determine whether E-gene primers and probe would amplify/detect specific viral or bacterial targets, bioinformatic analyses were performed via NCBI Blast (Table 5). Outside of the expected homology with SARS-CoV-1, results showed no significant homology (i.e., ≥ 80%).

Table 5. List of Organisms analyzed *in silico* for potential cross reactivity with the NEcov19 RT-PCR Assay

Pathogen	Strain	GenBank Accession#	%Homology Forward Primer	%Homolog Reverse primer	% Homolog Probe
Human coronavirus 229E	Seattle/USNSC9724/2018	MN369046.1	46.1%	40.9%	30.8%
Human coronavirus OC43	MDS12	MK303623.1	46.1%	40.9%	34.6%
Human coronavirus HKU1	SI17244	MH940245.1	38.5%	40.9%	42.3%
Human coronavirus NL63	Seattle/USNSC0768/2019	MN306040.1	42.3%	45.4%	42.3%
SARS CoV-1	Urbani isolate icSARS-C7-MA	MK062184.1	100%	100%	100%
Adenovirus C1	SG09/HAdvC1/2016	MN513345.1	38.5%	40.9%	38.4%

MERS CoV	Hu/Riyadh-KSA1801382/2018	MN723544.1	38%	40.9%	38.4%
Human Metapneumovirus (hMPV)	HMPV/Seattle/USA/SC0380/2019	KC562241.1	34.6%	40.9%	0%
Parainfluenza virus 1	HPIV1/USA/38078 A/2011	KF530203.1	0%	40.9%	38.4%
Parainfluenza virus 2	HPIV2/Los Angeles/USA/C HLA18/2016	MK167027.1	38.4%	40.9%	38.4%
Parainfluenza virus 3	HPIV3/Seattle/USA/SC9406/2019	MN306052.1	46.1%	50%	38.4%
Parainfluenza virus 4	HPIV4b/Seattle/USA/SC9597/2019	MN306058.1	50%	38%	47.6%
Influenza A	H3N2 A/Kitakyushu/ 159/93 PB1	AF037418.1	34.6%	40.9%	30.8%
Influenza B	B/lowa/14/2017	CY236691.1	34.6%	45.4%	26.9%
Enterovirus EV68	USA/CA/2014-RGDS-1025	MK681490.1	57.7%	40.9%	34.6%
Respiratory syncytial virus	RSV-A/US/BID-V8392/2003	MG027862.1	50%	40.9%	38.4%
Rhinovirus	Rhinovirus 59 strain 16-J2	KY629935.1	42.3%	54.5%	38.4%
Chlamydia pneumoniae	AR39	AE002161.1	42.3%	77.2%	50%
Haemophilus influenzae	10P129H1	CP029620.1	50%	59.0%	53.9%
Legionella pneumophila	Albuquerque 1 (D-7474)	CP021286.1	50%	59.0%	50%
Mycobacterium tuberculosis	TCDC11	CP045728.2	0%	63.6%	50%
Streptococcus pneumoniae	R6CIB17	CP038808.1	50%	50%	53.9%
Streptococcus pyogenes	1085	CP047120.1	53.9%	59.0%	46.2%
Bordetella pertussis	A340	CP033420.1	0%	63.6%	0%
Mycoplasma pneumoniae	M129	U00089.2	46.2%	46.2%	46.2%
Influenza C	C/Ann Arbor/1/50	AB126192.2	46.2%	36.4%	30.8%
Parechovirus	A isolate CBC	MK904606.1	42.3%	59.0%	42.3%
Candida albicans	TIMM1768	CP032012.1	57.6%	59.0%	50%
Corynebacterium diphtheriae	TH1526	CP038504.1	53.8%	54.5%	46.2%
Legionella non-pneumophila	*Legionellaceae*	TAXID 445	57.6%	53.9%	61.5%
Bacillus anthracis	MCCC 1A02161	CP031642.1	61.5%	73.6%	53.9%
Moraxella catarrhalis	MC6	CP010901.1	50%	59.0%	42.3%
Neisseria elongata	M15910	CP031255.1	46.2%	54.5%	61.5%
Neisseria meningitidis	95-134	CP021725.1	46.2%	63.6%	42.3%
Pseudomonas aeruginosa	VIT PC9	CP048791.1	50%	77.2%	46.2%
Staphylococcus epidermidis	1457	CP020463.1	53.9%	59.0%	61.5%
Streptococcus salivarius	57.1	CP002888.1	69%	54.5%	46.2%
Chlamydia psittaci	Ful127	CP033059.1	50%	59.0%	46.2%
Coxiella burnetii	RSA439	CP040059.1	61.5%	54.5%	46.2%
Staphylococcus aureus	FPR3757	CP000255.1	53.9%	63.6%	50%

Based on the *in silico* analysis, only SARS-CoV-1 may cross-react with the NEcov19 RT-PCR Assay. This homology is expected as SARS-CoV-1 is in the same subgenus (Sarbecorvirus) as SARS-CoV-2. In addition, SARS-CoV-1 is not known to be currently circulating in the human population, therefore is unlikely to be present in patient respiratory samples during the current emergency.

ii. Wet testing cross-reactivity

Cross-reactivity of the NEcov19 RT-PCR Assay was also evaluated by testing 33 independent nasopharyngeal swab (NPS) specimens that had resulted as positive on the FDA-cleared BioFire FilmArray respiratory pathogen (RP) panel. This set of NPS specimens represented 10 pathogens including 3 common human coronaviruses. None of these NPS specimens tested positive by the NEcov19 RT-PCR Assay (Table 6). The validity of each NPS sample was confirmed by a positive RNase P internal control result.

Table 6. Results of NEcov19 RT-PCR Assay on NPS Samples Containing Relevant Respiratory Viral Pathogens.

FilmArray RP Positive Analyte	# NPS Specimens	# NEcov19 Positives
Adenovirus	1	0
Coronavirus HKU1	8	0
Coronavirus NL63	2	0
Coronavirus OC43	3	0
Human metapneumovirus	6	0
Influenza A (H1)	5	0
Influenza B	1	0
Parainfluenza virus 4	1	0
Rhino/enterovirus	3	0
RSV	3	0
Total	33	0

4) Extraction Equivalency:

Equivalency of the following three extraction methods was evaluated by testing dilution series using the NEcov19 RT-PCR Assay:

- Automated extraction using the Roche MagNA Pure Total Nucleic Acid Kit with External Lysis Buffer on the Roche MagNA Pure Compact instrument.
- Automated extraction using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit on the ThermoFisher KingFisher Flex platform.
- Manual extraction using the QiaAmp DSP Viral RNA Mini Kit

A panel of serial dilutions from a SARS-CoV-2-positive NPS in VTM specimen was tested in this study. The dilutions were extracted using the Roche MagNA Pure Total Nucleic Acid Kit with External Lysis Buffer on the MagNA pure Compact extraction instrument (100 µL sample input volume with 50 µL elution volume), the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit on the ThermoFisher KingFisher Flex platform (400 µL sample input volume with 50 µL elution volume), and the QiaAmp DSP Viral RNA Mini Kit (280µL sample input volume with 60 µL elution volume), in parallel, in triplicate or duplicate, and the extracted

samples were tested using the NEcov19 RT-PCR Assay.

Table 7. Comparison of NEcov19 Assay Performance Across Three Extraction Methods

Dilution	MagMAX Pathogen RNA/DNA Kit on KingFisher Flex (400 µL input)		QiaAMP DSP Viral RNA Mini Kit (280 µL input)			Roche MagNA Pure Total Nucleic Acid Kit on Roche MagNA Pure Compact (100 µL input)		
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
$1:10^2$	17.5	17.6	18.1	18.0	18.4	20.6	20.5	20.6
$1:10^3$	20.7	20.5	21.2	21.3	21.2	23.8	23.8	29.2
$1:10^4$	23.2	23.7	25.0	23.8	27.4	27.3	27.2	26.9
$1:10^5$	27.7	27.2	28.3	28.3	28.6	30.9	33.5	30.8
$1:10^6$	30.9	31.2	31.5	32.1	32.1	33.8	34.0	34.1
$1:10^7$	34.3	34.0	36.0	35.0	35.6	39.3	37.6	NEG
$1:10^8$	38.1	NEG	38.8	NEG	NEG	36.0	NEG	NEG

Performance of the three extraction methods with the NEcov19 RT-PCR Assay appears to be similar.

5) ***Clinical Evaluation:***

The clinical performance evaluation of the NEcov19 RT-PCR Assay included testing of specimens from three sources:

- Patients under investigation for COVID-19 (PUIs; prospectively tested, high risk for SARS-CoV-2 infection)
- Heath care workers (HCW) under monitoring (prospectively tested, low risk for SARS-CoV-2 infection)
- Patients with respiratory symptoms tested by the FDA-cleared FilmArray respiratory pathogen panel (RPP) prior to the SARS-CoV-2 circulation in the U.S. (retrospectively tested, no risk for SARS-CoV-2 infection)

NPS specimens were tested from all three groups, with the addition of sputum samples for confirmed COVID-19 cases when available. A total of 72 specimens (69 NPS and 3 sputum specimens) were tested. Comparator method results with the CDC EUA assay were provided for specimens from PUIs. Specimens associated with low-risk health care worker monitoring were compared against an expected negative result, and repeated negative results were used for confirmation. No-risk patients with respiratory symptoms are compared against an expected negative result.

Overall, there was 100% (11/11) (95% CI: 74.1% - 100%) positive percent agreement between the NEcov19 assay and the comparator CDC assay for NPS specimens. There was 100% (58/58) (95% CI: 93.8% - 100%) negative percent agreement between the NEcov19 RT-PCR Assay and the comparator CDC assay or the expected negative results. There was also 100% (3/3) (95% CI: 43.8% - 100%) positive percent agreement between the NEcov19 assay and the known patient infected status for the three sputum samples tested.

To generate additional clinical performance data, 30 residual nasopharyngeal swab in VTM specimens with a positive result on the RMS cobas EUA test were tested by the NEcov19

Assay using the KingFisher extraction method. The NEcov19 Assay detected SARS-CoV-2 RNA in 30/30 of residual NPS in VTM specimens, resulted in 100% (95% CI: 88.6% - 100%) positive percent agreement with the RMS cobas EUA test. Ct values for both methods are shown in Table 8 below.

Table 8: NEcov19 Assay Clinical Performance in RMS cobas EUA Test-Positive NPS Specimens

Clinical NPS in VTM specimen	**RMS cobas EUA Test**		**NEcov19 Assay**	
	Target 1 Ct	**Target 2 Ct**	**E Ct**	**RNaseP Ct**
1	24.25	24.97	24.57	22.38
2	16.99	17.57	15.86	22.83
3	21.44	22.57	19.26	22.16
4	31.39	33.29	31.67	24.62
5	26.84	27.95	22.43	20.38
6	27.32	28.01	26.38	22.24
7	18.28	18.90	16.34	18.84
8	18.32	18.51	16.82	22.39
9	18.78	19.39	15.52	21.07
10	19.10	19.85	20.38	26.42
11	15.46	15.68	14.29	21.39
12	19.43	20.30	25.49	31.40
13	29.45	30.51	27.33	25.97
14	24.13	24.80	21.66	18.89
15	18.71	19.24	17.33	22.89
16	25.75	26.52	22.26	20.68
17	27.24	28.11	25.22	22.98
18	29.64	31.44	28.21	19.85
19	19.00	19.60	17.46	21.31
20	18.69	19.06	17.66	22.88
21	24.56	25.12	25.35	25.24
22	27.92	28.60	28.57	22.83
23	24.98	25.53	23.56	22.88
24	20.17	20.95	18.12	21.82
25	18.12	18.48	24.24	32.15
26	31.22	32.40	35.74	33.82
27	22.79	22.89	24.01	30.50
28	21.93	22.61	19.19	22.79
29	23.92	24.17	20.57	21.65
30	15.72	15.72	15.38	22.89

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by authorized laboratories;
- 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 diagnostic tests 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.