EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Gx HTKB SARS-CoV-2 Test
(RCA Laboratory Services LLC dba GENETWORx)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The Gx HTKB SARS-CoV-2 Test will be performed at RCA Laboratory Services LLC dba GENETWORx, located at 4060 Innslake Drive, Glen Allen, VA 23060 and 670 US 1, Iselin, NJ 08830, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The Gx HTKB SARS-COV2 Test is an *in vitro* diagnostic real-time reverse transcription polymerase chain reaction (rRT-PCR) assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in mid-turbinate and anterior nasal swab specimens collected from any individual, including from individuals without symptoms or other reasons to suspect COVID-19, using the NEST collection device when determined to be appropriate by a healthcare provider. Testing is limited to RCA Laboratory Services LLC dba GENETWORx, located at 4060 Innslake Drive, Glen Allen, VA 23060 and 670 US 1, Iselin, NJ 08830, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in mid-turbinate and anterior nasal swab 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 definitive cause of disease. Laboratories within the United States and its territories are required to report all test 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/or epidemiological information.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The Gx HTKB SARS-CoV-2 Test is a real time reverse transcription polymerase chain reaction (rRT-PCR) assay intended for the qualitative detection of nucleic acids from the SARS-CoV-2 in mid turbinate and anterior nasal swabs collected in NEST collection device from individuals suspected of COVID-19 by their healthcare provider and/or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection.

NEST collection device (Wuxi NEST Biotechnology Co., Ltd; Catalog number 202015) consists of a 5 mL vial with 2.5 mL VTM (Viral Transport Media) and one individually wrapped and sterile nasopharyngeal swab. GENETWORx provides the NEST devices to participating healthcare providers.

The assay is composed of two principal steps: (1) extraction of RNA from patient specimens on KingFisher Flex using MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit and (2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and real-time detection with SARS-CoV-2 specific probe labelled with FAM. The assay targets N1 region of the virus nucleocapsid gene (N1) and is designed for the detection of the SARS-CoV-2.

Amplification and detection are accomplished using TaqPath or TaqMan 1-step master mixes on the CFX384 Touch Real-Time PCR Detection System from BioRad. To ensure the absence of non-specific PCR inhibition in a sample, the assay targets human RNase P gene as an internal control. The probe is labelled with Quasar 670. A sample can be interpreted as negative only if the analysis of the RNase P gene indicates that amplification has occurred in the reaction tube and signal from SARS-CoV-2 target reporter dye is more than 40 Ct value.

Description of Test Steps:

1. *Sample aliquoting*: 200 µl of Viral Transport Medium (VTM) from each NEST device containing the collected MT or AN swab and up to 93 clinical samples are transferred to the 96 well deep well plate manually or using a Hamilton MicroLab Prep Instrument.
2. *Nucleic acid extraction*: using MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on KingFisher Flex, ThermoFisher. Volume used for nucleic acid resuspension is 75 µL.
3. *PCR set up*: PCR plates are created by dispensing 10 µl RT-PCR master mix to each well of a 384 well PCR plate followed by addition of 10 µl of extracted nucleic acid using the Eppendorf epMotion. Up to four 96-well sample plates can be combined in one 384 well PCR plate and the plate is sealed.
4. *Amplification and detection*: RT-PCR is performed on CFX384 Touch Real-Time PCR instrument (BioRad). The reaction is a one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and real-time detection with SARS-CoV-2 specific probe labelled with FAM.

   The RNA is reverse transcribed and amplified using 10 µL of TaqPath or TaqMan Master Mix (ThermoFisher) containing N1 and RNase P specific primers and probes. In the 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 probe, causing the reporter dye to separate from the quencher dye, generating 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 C1000 Touch and CFX Maestro Software.

INSTRUMENTS AND REAGENTS USED WITH THE TEST

The Gx HTKB SARS-CoV-2 Test is to be used with the following specific instruments, software and reagents:

Table 1. Gx HTKB SARS-CoV-2 Test Instrument, Software, and Reagents

Name	Vendor	Catalog ID or Model
KingFisher Flex Instrument	Thermo Scientific	5400630
BindIT Software for KingFisher	Thermo Scientific	5189009
MagMax Viral Pathogen Kit	ThermoFisher	A42352
Hamilton MicroLab Prep	Hamilton	Microlab Prep
Eppendorf epMotion 5075 TMX & 5075 LH, with epBlue Software	Eppendorf	epMotion 5075
BioRad CFX384 Real-Time System, C1000 Touch, with CFX Maestro Software	BioRad	CFX384 & C1000
TaqMan Fast Virus 1-Step Master Mix	ThermoFisher	4444432 4444434 4444436
TaqPath 1-Step Multiplex Master Mix (No ROX)	ThermoFisher	A28523

CONTROLS

Three external controls are required for each 96 well plate; therefore, each plate contains 93 samples plus one of each of the external controls. Additionally, there is an internal positive control to monitor each well amplification and verification of sample adequacy.

The following further describes the controls:

a) An external Positive Template Control (PTC) is needed to monitor the entire sample processing procedure, including the extraction and amplification. The PTC is prepared using ZeptoMetrix or Microbiologics inactivated SARS-CoV-2 in negative patient matrix diluted to 2,100 copies /mL (3xLoD) or positive pooled patient specimens of appropriate Ct value. One PTC for each 93 samples within a 96 well plate is used. PTC with a N1 Ct value in a range of 32-35 is acceptable for use in the assay.

b) An external Negative Template Control (NTC) is needed to monitor reagent contamination including extraction reagents; the NTC contains only nucleic acid elution buffer without a template. One NTC is required on every 96-well extraction plate. An NTC Ct value of >40 Ct for N and RP are acceptable.

c) Human Template Control (HTC) monitors extraction and amplification processes and reagent contamination for N1 gene/amplicon. It consists of buccal swabs in NEST

VTM collected from Medical Technologist volunteers confirmed negative for SARS-CoV-2. It also verifies performance of the RP assay on the plate. It is required on every 96-well extraction plate. An RP Ct value of <40 Ct and N Ct value >40 is acceptable. The HTC must be verified before use in clinical testing.

d) An internal Positive Control (IPC) monitors the extraction, amplification, and detection steps of each specimen and confirms the appropriate collection of the specimens. The human RNase P (RP) gene constitutes the IPC and is tested in every patient sample. The IPC is sensitive to possible inhibitors of SARS-CoV-2 virus detection. All negative samples must be positive for RNase P to be valid. A RP Ct value of <40 Ct is acceptable.

INTERPRETATION OF RESULTS

Assay Controls

Assessment of Gx HTKB SARS-CoV-2 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.

Refer to **Table 2** for Acceptance Criteria for Controls in Gx HTKB SARS-CoV-2 Test

Table 2. Acceptance Criteria for Controls in Gx HTKB SARS-CoV-2 Test.

CONTROL NAME	CONTROL ID	Acceptance Criteria	
		N1	RP
Positive Template Control	PTC	31-39 Ct	Detected/ Not Detected
No Template Control	NTC	ND	ND
Human Template Control	HTC	ND	< 40 Ct

ND=Not Detected

Clinical Specimens

For sample interpretation, the amplification graphs are reviewed prior to releasing results. Refer to **Table 3** for Interpretation of patient sample results for Gx HTKB SARS-CoV-2 Test.

Table 3. Interpretations of patient sample results

N1	RP	INTERPRETATION	RESULT	Action
< 40	Detected/ Not Detected	Sample is positive for SARS-CoV-2	Positive	Report the result
≥ 40	< 40	Sample is negative for SARS-CoV-2	Negative	Report the result
≥ 40	≥ 40	Failure in sample collection or procedure	Invalid	Repeat from extraction

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity:

A preliminary LoD study for the Gx HTKB SARS-CoV-2 Test was completed using

inactivated SARS-CoV-2 from Microbiologics (Lot # K2009C) at a stock concentration of 4.50E+09 copies/mL, which was serially diluted 1:10 twice followed by 1:3 serial dilutions in a negative Nest VTM patient matrix from Mid-turbinate (MT) nasal swab collections. Each serial dilution was extracted in triplicate and amplified in duplicate using TaqMan Fast Virus 1-Step Master Mix or TaqPath 1-Step Multiplex Master Mix on BioRad CFX384 Real-Time System. The preliminary LoD was defined as the lowest virus concentration at which positivity was observed for all six replicates (100% detection) using TaqMan Fast Virus 1-Step or TaqPath 1-Step Multiplex Master Mix. The preliminary LoD of SARS-CoV-2 detection from MT swab specimens was determined as 7.62E+02 copies/ml.

Table 4. Preliminary LoD results for both TaqPath and TaqMan Master Mixes

#	Concentration (cp/mL)	A. TaqPath Master Mix			B. TaqMan Master Mix		
		N1 Detected	RP Detected	% Positive	N1 Detected	RP Detected	% Positive
1	4.50E+07	6/6	6/6	100	6/6	6/6	100
2	1.50E+07	6/6	6/6	100	6/6	6/6	100
3	5.00E+06	6/6	6/6	100	6/6	6/6	100
4	1.67E+06	6/6	6/6	100	6/6	6/6	100
5	5.56E+05	6/6	6/6	100	6/6	6/6	100
6	1.85E+05	6/6	6/6	100	6/6	6/6	100
7	6.17E+04	6/6	6/6	100	6/6	6/6	100
8	2.06E+04	6/6	6/6	100	6/6	6/6	100
9	6.86E+03	6/6	6/6	100	6/6	6/6	100
10	2.29E+03	6/6	6/6	100	6/6	6/6	100
11	**7.62E+02**	**6/6**	**6/6**	**100**	**6/6**	**6/6**	**100**
12	2.54E+02	5/6	6/6	83.3	3/6	6/6	50
13	8.47E+01	2/6	6/6	33.3	0/6	6/6	0
14	2.82E+01	4/6	6/6	66.6	1/6	6/6	16.7
15	9.41E+00	0/6	6/6	0	0/6	6/6	0

An LoD confirmation study of the Gx HTKB SARS-CoV-2 Test was completed by running 20 replicate samples at three dilutions, at 2.29E+03, 7.62E+02, and 2.54E+02 cp/ml of the virus in NEST negative patient matrix along with controls per the assay SOP. The LoD was confirmed to be 7.62E+02 copies/mL. LOD testing supports the use of either TaqMan or TaqPath master mix as the data demonstrate equivalency.

Table 5. LoD Confirmation Results

Concentration (cp/mL)	TaqPath Master Mix		TaqMan Master Mix	
	%Positive (positive/tested)	N1 Ct Average	%Positive (positive/tested)	N1 Ct Average
2.29E+03	100% (20/20)	32.74	100% (20/20)	35.50
7.62E+02	**95% (19/20)**	**34.83**	**100% (20/20)**	**37.16**
2.54E+02	72.22% (14/18)*	35.75	55.00% (11/20)	38.02

**- 2 invalid samples*

2) <u>Inclusivity (Analytical Reactivity):</u>

To mitigate the possibility that currently known emerging variants and mutations might prove detectable by this assay at less than acceptable limits, the GISAID database for emerging

SARS-CoV-2 mutations was reviewed on 10/9/2023 and all commercially available synthetic variants were evaluated. *In silico* analysis indicated that the probe hybridization site is mostly affected, but wet lab assessment of Twist controls that include the most common mutations revealed that the mutations within the probe sequence did not impact the test's performance. In addition, mutations within the primer hybridization sites were observed at lower frequency. These mutations affect single bases and are not likely to severely impact the assay.

In the future, any concerns noted as the assay is assessed against any new, or emerging, variants or mutations will be immediately shared with the FDA.

3) Cross-Reactivity (Analytical Specificity):

Cross reactivity with microorganisms commonly found in mid-turbinate and anterior nasal swab samples was evaluated via *in silico* analysis using published genome sequences and the sequences of Gx HTKB SARS-CoV-2 Test's primers and probe. There were 16 microorganisms identified that may interfere with amplification of SARS-CoV-2 nucleic acid in upper respiratory samples due to having ≥ 80% sequence homology to the assay's primers or probe (refer to Table 6). These pathogens were studied further in Microbial Interference studies.

Table 6. *In silico* cross-reactivity analysis results between primers-probe of the assay and microorganisms present in upper respiratory samples

Group	Organism	Sequence accession	≥ 80% Homology
Pathogen of interest	SARS-CoV-2	NC_045512.2	Yes
Similar, high priority pathogens	Human coronavirus 229E	AF304460.1	Yes
	Human coronavirus HKU1	NC_006577.2	No
	Human coronavirus NL63	NC_005831.2	No
	Human coronavirus OC43	NC_006213.1	Yes
	SARS-CoV-1	NC_004718.3	Yes
	MERS-coronavirus	NC_019843.3	No
Likely present, high priority pathogens	Adenovirus	KF268207.1	No
	Human metapneumovirus	NC_039199.1	Yes
	Human parainfluenza virus 1	NC_003461.1	No
	Human parainfluenza virus 2	NC_003443.1	No
	Human parainfluenza virus 3	NC_001796.2	No
	Human parainfluenza virus 4a	NC_021928.1	No
	Influenza A	NC_007382.1, NC_007374.1, NC_007381.1, NC_007375.1, NC_007380.1, NC_007376.1, NC_007377.1, NC_007378.1	No
	Influenza B	NC_002205.1, NC_002204.1, NC_002206.1, NC_002210.1, NC_002209.1, NC_002207.1, NC_002211.1, NC_002208.1	No

Group	Organism	Sequence accession	≥ 80% Homology
	Enterovirus 68	NC_038308.1	Yes
	Respiratory syncytial virus	MK733766.1	No
	Bordetella pertussis	CP011448.1	Yes
	Candida albicans	NC_032089.1, NC_032090.1, NC_032091.1, NC_032092.1, NC_032093.1, NC_032094.1, NC_032095.1, NC_032096.1	Yes
	Chlamydia pneumoniae	NC_005043.1	Yes
	Haemophilus influenzae	CP000672.1	Yes
	Legionella pneumophila	NZ_CP015941.1	Yes
	Mycoplasma pneumoniae	NZ_LR214945.1	Yes
	Pseudomonas aeruginosa	CP007224.1	Yes
	Rhinovirus A	NC_038311.1	No
	Rhinovirus B	NC_038312.1	No
	Rhinovirus C	NC_009996.1	No
	Staphylococcus epidermis	NZ_CP035288.1	Yes
	Streptococcus pneumoniae	CP027540.1	Yes
	Streptococcus pyogenes	AE014074.1	Yes
	Streptococcus aureus	CP066093.1	Yes

Organisms in gray shaded boxes were further tested for microbial interference.

4) Microbial Interference:

Sixteen microorganisms from the *in silico* cross-reactivity analysis were found to have ≥ 80% homology to one of the primers or probes in the SARS-CoV-2 primers/probe set (refer to Table 6). A microbial interference study was performed to further evaluate potential interference of these organisms. To evaluate microbial interference, samples in triplicate were prepared by spiking inactivated SARS-CoV-2 at 3X LoD and with the potentially interfering microorganisms at high concentration in negative swab in Nest matrix. One sample was tested without microorganism to serve as a reference. Results showed that high concentration of microorganisms that are commonly found in respiratory specimens and have ≥ 80% homology to the SARS-CoV-2 primers or probes do not interfere with the detection of SARS-CoV-2 when present at low concentration.

5) Endogenous/Exogenous Interference Evaluation:

Interference with potentially interfering endogenous and exogenous substances commonly found in upper respiratory samples was evaluated to assess their impact on assay performance. Inactivated SARS-CoV-2 was serially diluted to a concentration of 3X LoD in negative NP Nest patient matrix, and the Interfering Substances were spiked into the SARS-CoV-2 sample at the concentrations shown in Table 7. One sample prepared with PBS instead of interfering substance served as a reference. Triplicate samples were prepared. No significant interferences were detected at the concentrations tested. Testing with Nasacort Nasal Spray showed an increase in the N1 Ct value of 1.25. Therefore, the presence of Nasacort Nasal Spray may potentially interfere with the detection of SARS-CoV-2 by Gx HTKB SARS-CoV-2 in very low positive samples.

Table 7. Endogenous/Exogenous Interference Study Results

Interference Substance (concentration)	with SARS-CoV-2 virus	
	RNaseP gene positive/tested	N1 gene positive/tested
Sore Throat Spray (5% v/v)	3/3	3/3
Mouth Wash (5% v/v)	3/3	3/3
Cough Syrup (5% v/v)	3/3	3/3
Nicotine (.03 mg/ml)	3/3	3/3
Toothpaste (.5% v/v)	3/3	3/3
Human Genomic DNA (10ng/uL)	3/3	3/3
Afrin Original Nose Spray (15% v/v)	3/3	3/3
Cough Lozenges (3 mg/mL)	3/3	3/3
Nasacort Allergy 24HR (glucocorticoid) Nasal Spray (10% v/v)	3/3	3/3
Whole Blood (K_2EDTA collection tube) (2.5%)	3/3	3/3
PBS (10% v/v)	3/3	3/3
Mucin (2.5 mg/mL)	3/3	3/3

6) Specimen Stability:

Specimen stability was evaluated to support claims for transportation and storage of MT samples in Nest VTM. Specimens were stable for a total of 142 hours including collection (2 hours), shipping under summer or winter conditions (56 hours) and storage at room temperature or refrigerated upon arrival (84 hours).

7) Clinical Evaluation (Symptomatic Patient Group):

The clinical evaluation study was performed on 209 (mid-turbine and anterior nares) swab samples collected in NEST collection device from patients suspected of COVID-19 by their healthcare provider. Samples were collected at multiple collection sites served by RCA Laboratory Services LLC dba GENETWORx. Samples (n=201) received for routinary testing between August 3 and August 18, 2023, that meet inclusion/exclusion criteria were

prospectively included in the study without any other pre-selection. The study also included eight positive samples (7 MT and 1 AN) collected between August 29 and September 9, 2023, which were added to increase the number of AN and MT positive samples. Following the clinical testing, all samples were archived by freezing (-70ºC) and once sufficient samples were obtained, the samples were tested by the Gx HTKB SARS-COV2 Test and the comparator test. R&D personnel conducting the study were blinded as to the production test data to avoid bias and the comparator and candidate tests runs were performed by separate technologists in blind fashion.

The study included 141 anterior nasal and 68 mid-turbinate swab samples collected in NEST device. According to the comparator test, there were 30 AN and 31 MT positive samples for a total of 61 positive samples, including low positive samples, and there were 111 AN and 37 MT negative samples for a total of 148 negative samples. Testing with the Gx HTKB SARS-COV2 Test resulted in no false negative results and two false positive results in the AN specimens and no false negative or false positive results in the MT specimens.

The calculated OPA, PPA, and NPA for the combined AN and MT samples were 99.0% (95%CI = 99.6% - 99.7%), 100.0% (95%CI = 94.1% - 100%), and 98.6% (95%CI = 95.2% - 99.6%), respectively, indicating that they all met the ≥ 95% agreement pre-specified acceptance criteria. Table 8 shows the results by sample type and overall.

Table 8. Clinical Evaluation Summary - Symptomatic

			Comparator Test		**Gx HTKB SARS-COV2 Test**	
			Positive	**Negative**	**PPA(%) 95% CI**	**NPA(%) 95% CI**
Gx HTKB SARS-COV2 Test	AN	Positive	30	2	100 88.6-100	98.2 93.7-99.5
		Negative	0	109		
	MT	Positive	31	0	100 89.0-100	100 90.6-100
		Negative	0	37		
	Overall	Positive	61	2	100 94.1-100	98.6 95.2-99.6
		Negative	0	146		

Clinical Evaluation (Screening of Individuals Without Symptoms Group):

The clinical evaluation study for screening of individuals without symptoms or other reasons to suspect COVID-19 was performed on 48 AN and 88 MT nasal samples collected consecutively over several time periods. Samples were tested by the Gx HTKB SARS-COV2 Test method and the comparator test. There were 25 positive and 111 negative sample results by the comparator test. The Gx HTKB SARS-CoV-2 test produced one False Positive result. The OPA, and PPA met the ≥95% acceptance criteria, including the lower bound of the two-sided 95% confidence interval >76% for the PPA. Additionally, the NPA was ≥98% with its lower bound of the two-sided 95% confidence interval as >95% showing that acceptance criteria were also met (Table 9).

Table 9. Clinical Evaluation Summary - Asymptomatic

		Comparator Test	
		Positive	**Negative**
Gx HTKB SARS-COV2 Test	**Positive**	25	1
	Negative	0	110
OPA: 96.3%, 95%CI = 96.0%-99.9% PPA: 100% , 95%CI = 86.7%-100% NPA: 99.1% , 95%CI = 95.1%-99.8%			

Limitations

- 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.
- 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.

WARNINGS

- For prescription use only.
- For in vitro diagnostic use.
- For use under Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product 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 declaration is terminated or authorization is revoked sooner.