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GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT

Instructions for Use

GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT

Catalog # GK-1000T-01

1000 reactions

For In-vitro Diagnostic (IVD) Use

Rx Only

For use under Emergency Use Authorization (EUA) Only

Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency.

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1. Intended Use

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT is an in vitro diagnostic real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, mid-turbinate, and nasal swabs) collected from individuals suspected of COVID-19 by their healthcare provider. Testing in the United States is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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

2. Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organisation (WHO) in December 2019. Chinese authorities identified a novel coronavirus SARS-CoV-2 (COVID-19 previously called 2019-nCoV) which has resulted in thousands of confirmed human infections worldwide, including the United States. Cases of asymptomatic infection, mild and severe respiratory illness and deaths have been reported. Patients can become infected with SARS-CoV2 virus by person-person contact (through contact with a contaminated environment or person).

On January 31, 2020, US Health and Human Services Secretary declared a public health emergency for the United States to aid the nation's healthcare community in responding to SARS-CoV-2. The emergence and rapid spread of SARS-CoV-2 to numerous areas throughout the world, has necessitated preparedness and response in laboratories, as well as health care and other areas of society in general. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies and surveillance studies.

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

The term "qualified clinical laboratory personnell" requires that all users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use.

3. Principles of the Procedure

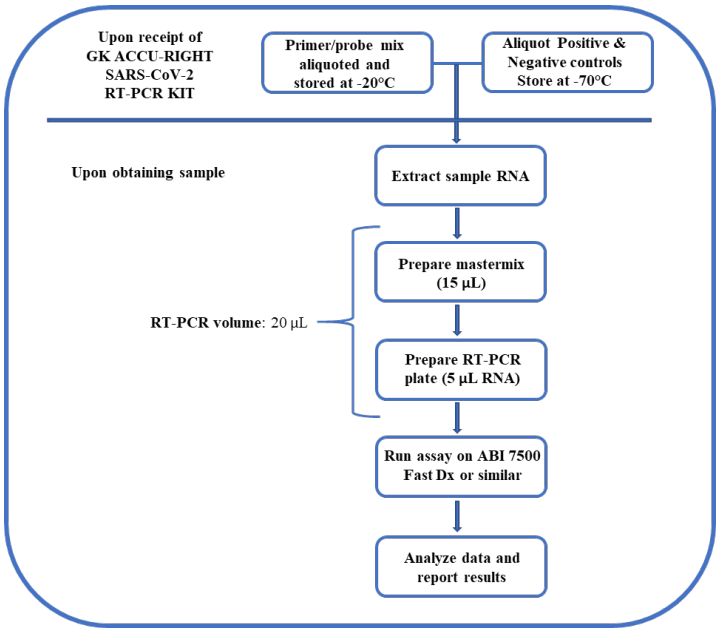
The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT is an in vitro diagnostic test based on Real-Time PCR technology, developed for specific detection of SARS-CoV-2 viral RNA. The probe system is based on the standard hydrolysis probe system known as TaqMan® Technology. The SARS-CoV-2 specific targets and the internal control are labelled with FAM and run in individual reactions.

Nucleic acid from patient samples are extracted and purified as described in the procedural steps. Selective amplification of target nucleic acid from the sample is achieved by reverse transcription of the SARS-CoV-2 RNA as well as the host specific RNase P RNA and subsequent PCR amplification using the target-specific forward and reverse primers.

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT utilizes primers and probes targeting RNA from the SARS-CoV-2 coronavirus virus nucleocapsid phosphoprotein (N) gene. The panel is designed for specific detection of two unique regions (N1 and N2) of the SARS-CoV-2 (two primer/probe sets). The primer and probe sets are based on the United States Centers for Disease Control and Prevention (US CDC) 2019-nCoV Real-Time RT-PCR Diagnostic Panel. An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel. The internal control RP will serve as an endogenous nucleic acid extraction control present in all properly collected patient simples and serves as both an extraction control and an internal amplification control. All three targets are amplified in individual reactions.

RNA isolated from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4. 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), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument.

Figure 1. GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT workflow



4. Product Description (Materials Required and Provided)

Each GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT consists of the following components:

Table 1. GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT components

GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT		
KIT Components	**1000 test/kit**	**Storage Conditions (°C)**
GK SARS-CoV-2 qPCR Master Mixture[1]	3 x 1700 µL	-20 °C
GK SARS-CoV-2 Primers & Probes Mixture 1[2]	1 x 1600 µL	-20 °C
GK SARS-CoV-2 Primers & Probes Mixture 2[3]	1 x 1600 µL	-20 °C
GK SARS-CoV-2 Primers & Probes Mixture 3[4]	1 x 1600 µL	-20 °C
GK SARS-CoV-2 Positive Control[5]	800 µL	-20 °C
GK SARS-CoV-2 Negative Control[6]	800 µL	-20 °C

1. GK SARS-CoV-2 qPCR Master Mixture: This-HCL, MgCL2 and dNTPs, RT Enzyme, Taq Enzyme
2. GK SARS-CoV-2 Primers & Probes Mixture 1: Primer pairs and probes for amplification and detection of N1 gene (see table 2).
3. GK SARS-CoV-2 Primers & Probes Mixture 2: Primer pairs and probes for amplification and detection of N2 gene (see table 2).
4. GK SARS-CoV-2 Primers & Probes Mixture 3: Primer pairs and probes for amplification and detection of RNaseP gene to verify sample quality (see table 2).
5. GK SARS-CoV-2 Positive Control: Positive control for all three targets.
6. GK SARS-CoV-2 Negative Control: Negative control for targets. It consisting of Nuclease free water, is needed to verify the master Mixture is free of contamination. This control should be included with each run.

Table 2. Primers and Probes sequences included in the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT

Target	Name	Description	Oligonucleotide sequence (5' →3')	Label
GK SARS-CoV-2 Primers & Probes Mixture 1	2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None
	2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None
	2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC BHQ1-3'	FAM-BHQ-1
GK SARS-CoV-2 Primers & Probes Mixture 2	2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None
	2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None
	2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AGBHQ1-3'	FAM-BHQ-1
GK SARS-CoV-2 Primers & Probes Mixture 3	RP-F	RNase P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None
	RP-R	RNase P Reverse Primer	r 5'-GAG CGG CTG TCT CCA CAA GT-3'	None
	RP-P	RNase P Probe	5'-FAM TTC TGA CCT GAA GGC TCT GCG CG BHQ-1-3'	FAM-BHQ-1

5. Human Specimen Control (HSC) not provided (optional):

- While the HSC is optional for use, if it is included in a test run its results must be incorporated in the Quality Control for such a test run. The HSC control consists of the following alternative materials:
- Negative human specimen material: Laboratories may prepare a volume of human specimen material (e.g., human sera or pooled leftover negative respiratory specimens) to extract and run alongside clinical samples as an extraction control. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.
- Contrived human specimen material: Laboratories may prepare contrived human specimen materials by suspending any human cell line (e.g., A549, Hela or 293) in PBS. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.

6. Required Equipment and Consumables (Not Provided)

- QIAamp® Viral RNA Mini Kit for RNA extraction (Qiagen; Cat No./ID: 52906).
- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument.
- PCR Workstation or Biological Safety Cabinet
- Benchtop refrigerated microcentrifuge
- Vortex mixer
- Adjustable micropipettes (2 or 10 µl, 200 µl and 1000 µL)
- Adjustable multichannel micropipettes (5-50 µL)
- Racks for 1.5 mL microcentrifuge tubes
- Cold blocks
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- Aerosol barrier pipette tips with filters
- Disposable powder-free gloves and dedicated laboratory coat
- The following methods of surface decontamination are provided as suggestions:
    - 30-watt UV lamp to eliminate environmental contamination
    - 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach) or 20% (v/v) bleach solution (2.0% w/v sodium hypochlorite in water)
    - DNAZap™ (Fisher Scientific; cat. #21-236-28), or equivalent
    - RNAse Away™ (Fisher Scientific, cat. no: 21-236-21) or equivalent
- 0.1 mL PCR reaction plates or 8-Tube Strip. Depending on the Real-Time PCR instrument to be used.
- MicroAmpTM Optical 8-cap Strips
- Molecular grade water nuclease free
- Plate seal
- 70% ethanol

7. Reagent Storage, Handling and Stability Conditions

- Store the kit at -20°C.
- Store liquid HSC control materials at ≤ -20°C.
- Protect fluorogenic probes from light.
- Limit freeze/thaw cycles for kit reagents to 5 freeze/thaw cycles.

- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.
- Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.
- Always check the expiration date prior to use. Do not use expired reagents.
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is 12 months.

8. Warnings and Precautions

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT workflow should be performed by qualified and trained staff to avoid the risk of erroneous results.

8.1 General Considerations

- For use only under FDA emergency use authorization.
- For in vitro diagnostic use (IVD).
- For prescription use only.
- The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT has not been FDA cleared or approved.
- The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests.
- The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT 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.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Specimen processing should be performed in accordance with national biological safety regulations.
- Laboratory staff should be familiar with the protocol and instruments used.
- Handle all specimens as infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Follow standard precautions, all patient specimens and positive controls should be considered potentially infectious and must be handled under a laminar airflow hood or biological safety cabinet.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Use separate areas for the preparation of patient samples and controls to prevent false positive results.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Follow necessary precautions when handling specimens. Use personal protective equipment consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.

- Dispose of waste in compliance with the local, state, and federal regulations.
- Please consult the safety data sheet (SDS) before using this kit, which is available on request.
- Please consult the relevant Instruction for Use (IFU) and Materials Safety Data Sheet (SDS), available from the manufacturer, before using your chosen IVD extraction kit/system.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Reagents must be stored and handled as specified in the kit instructions.
- Always check the expiration date of the kit prior to use. Do not use expired reagent.
- Do not substitute or mix reagent from different kit lots or from other manufacturers.
- Store primer/probes and the master mixture of enzymes at appropriate temperatures (see package inserts). Do not use reagents beyond their expiration dates.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

8.2 Preventing Contamination

- Amplification technologies such as PCR are sensitive to contamination with amplifiable materials such as positive control materials or PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Therefore, follow the following recommendations to mitigate the risk of contamination:
  - GK SARS-CoV-2 Positive and Negative Controls should be opened and processed away from samples and kit components to avoid cross-contamination.
  - Separate work areas should be used for:
    - Receipt and handling of specimen preparation (pre-processing steps)
    - Nucleic acid extraction
    - Reagent preparation (e.g., preparation of RT-PCR master mix; No amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition area)
    - Nucleic acid addition
    - Instrumentation (e.g., thermocyclers)
  - Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
  - Change aerosol barrier pipette tips between all manual liquid transfers.
  - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
  - Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. microcentrifuge tubes, pipette tips) for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
  - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
  - The workflow should always be from the clean area to the dirty area.
  - Change gloves between samples and whenever contamination is suspected.
  - Keep reagent and reaction tubes capped or covered as much as possible.
  - When mixing reagents by pipetting up and down, this should be done with a volume roughly equal to 50% of the total component volume.
  - Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.

- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as ethanol 70%, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination.

- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken.

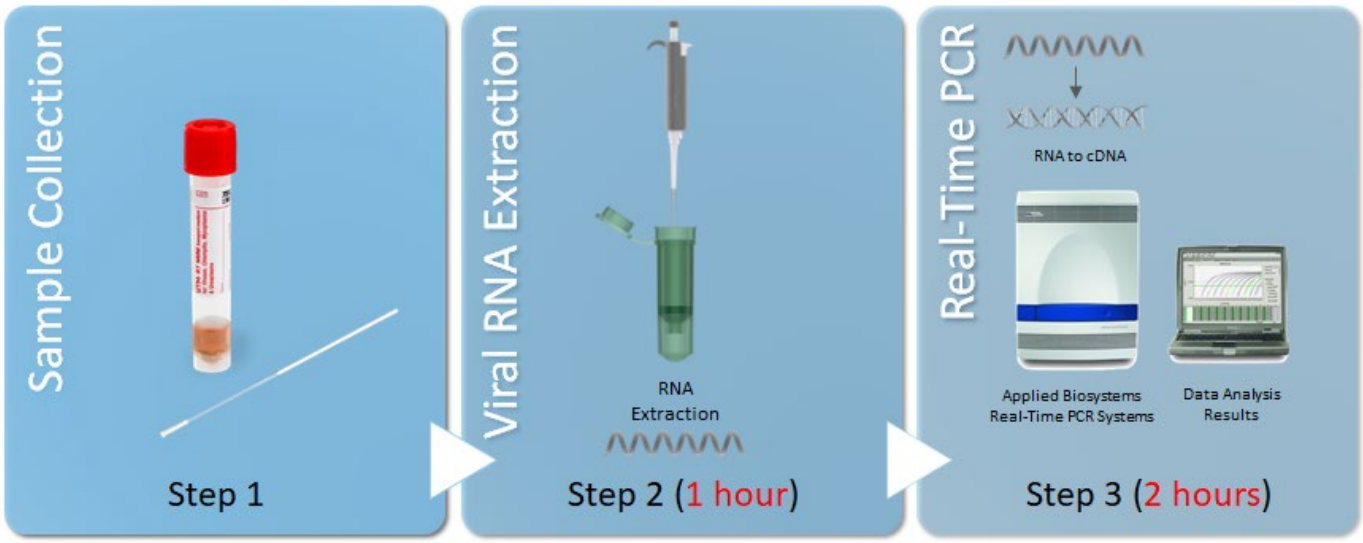
9. Quality Control

Tests should be performed using strict quality controls and quality assurance procedures. Following these Instructions for Use will help minimize the possibility of falsely positive amplification.

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT contains positive and negative controls that should be included in each test for a correct result interpretation. The kit also contains primers and probes for the amplification of the human RNAse P gene to monitor functionality of extraction and reverse transcription reagents.

10. GK ACCU-RIGHT SARS-CoV-2 RT-PCR assay procedure

Figure 2. Schematic View of Assay Procedure



10.1 Specimen Collection, Handling and Storage

- Adequate, appropriate specimen collection, transport and storage are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results.
- The recommended sample type for GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT is an upper respiratory specimen (such as nasopharyngeal, oropharyngeal, mid-turbinate, or nasal swab). Swabs should be collected in viral transport media. The viral transport media volume validated with this test is 3 mL.
- Samples should be collected into sterile, labeled tubes, and shipped at 2°C to 8°C on frozen gel packs.
- Samples that have not been pre-approved for testing and those that are labeled improperly will not be tested until the required information is obtained.
- Follow specimen collection devices manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are

not recommended. Place swabs immediately into sterile tubes containing 2-3 mL of viral transport media. In our LOD and clinical study a viral transport media based on the CDC recommendations was validated.

- Swabs were collected into 3 mL viral transport media (VTM based on the CDC recommendations for viral transport media) and tested with the EUA authorized
- Store specimens at 2-8 °C for no longer than seventy-two (72) hours. For prolonged storage, freeze at ≤ -70°C.
- Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or at -70°C if stored longer than 4 hours.
- Residual samples and the extracted nucleic acid should be stored at -70°C. Only defrost the amount of sample or extracted RNA that will be tested in a single day. Do not freeze/defrost extracts more than once before testing.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.
- Sample rejection criteria:
  - ✓ Samples are not kept at 2-8 °C (3 days) or frozen at a higher temperature than -70 °C.
  - ✓ Labeling or incomplete documentation of the sample.
  - ✓ Inappropriate sample type (e.g: sample types other than those listed in the intended use of this test).

For more information, refer to: Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019nCoV/guidelines-clinical-specimens.html.

10.2 Reagent preparation

Note: Change gloves often and when necessary to avoid contamination.

GK SARS-CoV-2 Primers & Probes Mixture Sets

- Cautions: these reagents should only be handled in a clean area and stored at appropriate temperatures in the dark. Freezing and thawing cycles should be avoided. Keep cold when defrosted. Make single-use aliquots and store at -20°C in a frost-free freezer.

GK SARS-CoV-2 Positive Control

- Cautions: This reagent should be handled with caution in an area dedicated to the handling of nucleic acids to avoid possible contamination.
- This reagent is used to evaluate the performance of RT-PCR tests. Before extraction, we recommend making single-use aliquots of 70 µL and storing at -20°C.
- After extraction the aliquots extracted from the positive control must be stored as described in the Nucleic Acid Extraction chapter of this instruction for use.
- Defrost a single positive control aliquot for each experiment and keep it on ice until added to the plate.
- GK SARS-CoV-2 Positive Control also contains human DNA that serves as a positive control for the RP assay.

10.3 Nucleic Acid Extraction

Performance of RT-PCR amplification-based tests depends on the quantity and quality of sample the RNA template. Perform Nucleic Acid extraction using the QIAamp Viral RNA Mini Kit (Catalog No. 52906) according to the following procedure.

Qiagen QIAamp® DSP Viral RNA Mini Kit:

- In an area separate from sample accessioning and RT-PCR reaction set-up dilute 70 µL of the Negative Control contained in the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT with 70 µL of nuclease free water.
- In an area separate from sample accessioning and RT-PCR reaction set-up dilute 70 µL of the Positive Control contained in the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT with 70 µL of nuclease free water for a final concentration of 10 copies/µL.

- Utilize 140 µL of controls and patient samples for the extraction. Follow the extraction instructions provided in the *Qiagen QIAamp® DSP Viral RNA Mini Kit*. Elute RNA with 60 µL of buffer.
- Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or at -70°C if stored longer than 4 hours.
- Keep the residual sample and the extracted nucleic acid store immediately at -70°C. Just defrost the amount of RNA extracted that will be analyzed in a single day. Do not freeze/defrost extracts more than once before testing.

10.4 Reaction Master Mix and Plate Setup

Notes: Run configuration can vary with the number of specimens and work-day organization.
GK SARS-CoV-2 Negative and Positive Controls must be included in each run.
Optionally, laboratories may consider including a Human Sample Control (HSC) as an additional extraction control not provided with the kit.

1) In the clean area of the reagent preparation room, place the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT reagents on a cold block. Keep cool during preparation and use.
2) Defrost the reaction master mixture and the primer/probe before use.
3) Mix the master mixture and the primer/probes by inversion 3-5 times.
4) Briefly centrifuge (3-5 seconds) the master mixture and primers/probes and return to the cold block.
5) Label a 1.5 mL microcentrifuge tube for each primer/probe set.
6) Determine the number of reactions (N) run per test including GK SARS-CoV-2 Controls, and the HSC (as applicable). Use the following guide to determine N considering additional volume to compensate for volume lost due to pipetting:
   • If the number of samples (n) including the controls is equal to 1 to 14, then N = n + 1
   • If the number of samples (n) including controls is 15 or greater, then N = n +2
7) For each set of primer/probe, dispense reagents in their respective labeled 1.5 ml microcentrifuge tube and mix by pipetting up and down. Don't vortex.
8) Centrifuge for 5 seconds to collect the contents at the bottom of the tube and then place the tube on a cold rack.

Table 3. GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT RT-PCR Master Mix Preparation

Steps	Reagents	Reagent Vol (µL) 1 Reaction	Reagent Vol (µL) N reactions
1	GK SARS-CoV-2 qPCR Master Mixture	5.0	N x 5.0
2	GK SARS-CoV-2 Primers & Probes Mixture	1.5	N x 1.5
3	RNase free water	8.5	N x 8.5
	Total volume	15.0	N x 15.0

Note: Where N is the number of controls and samples to evaluate plus 1 if it is up to 14 samples and more 2 if they are more than 15 samples.

9) Install tubes or plates of reaction strips in a 96-well cooler rack.
10) Dispense 15 µL of each master mixture into the appropriate wells.
11) Prior to moving to the nucleic acid handling area, prepare the Negative Control reaction/s in the assay preparation area:
    - Pipette 5 µL of GK SARS-CoV-2 Negative Control in the Negative Control test tub. Cover the Negative Control well/s before proceeding.
12) Cover the entire reaction plate or tubes and move to the nucleic acid sample handling area.

10.5 Template Addition

1) Gently shake the nucleic acid sample tubes in vortex for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube on ice or in a cold rack.
3) Carefully pipette 5 µL of each sample into each sample tube. Keep other sample tubes closed during addition. Change pipette tips after each addition.
4) If necessary, add 5 µL of sample extracted from Human Sample Control (HSC) extracted sample to the HSC wells.
5) Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

10.6 Assay Control Addition

1) Gently vortex the GK SARS-CoV-2 Positive Control for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube and then place on ice or in a cold rack.
3) Carefully pipette 5 µL of GK SARS-CoV-2 Positive Control into the positive control tube.
4) NOTE: If using 8-tube strips, label the TAB of each strip to indicate the position of the sample. Do not label the reaction tube caps as the labels with disappear!
5) Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack. NOTE: If using 96-well plates, centrifuge the plates for 30 seconds at 500 x g, 4°C.

10.7 Instrument set up

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT is to be used with the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Systems (software version 1.4)

Important: All laboratories must ensure that the instruments used have been installed, calibrated, and maintained in accordance with the manufacturer's instructions and recommendations.

- Clean and decontaminate all work surfaces, pipettes, centrifuges and other equipment prior to use. Decontamination agents should be used including 70% ethanol, and DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.
- Turn on Applied Biosystems™ 7500 Fast Dx Real-Time PCR Systems
- Perform the board configuration and select the cycle protocol on the instrument.
- Instrument configuration:
  - Detector (FAM); Quencher (None).
  - Passive reference: (Rox).
  - Run mode: (Standard).
  - Sample volume: (20 µL).
- Please refer to Applied BiosystemsTM 7500 Fast Dx Real-Time PCR system Relative Standard Curve and comparative Ct Experiments (Applied Biosystems manual) for programming the Real-Time PCR Instrument.
- Enter the following amplification program:

Table 4. GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT RT-PCR Thermocycling Parameters

	Stage	Cycle Repeats	Acquisition	Temperature (°C)	Time (min:sec)
UNG Incubation	Hold	1	-	25	02:00
Reverse Transcription	Hold	1	-	50	15:00
Polymerase Activation	Hold	1	-	95	02:00
Denaturation	Cycling	42	-	95	00:15

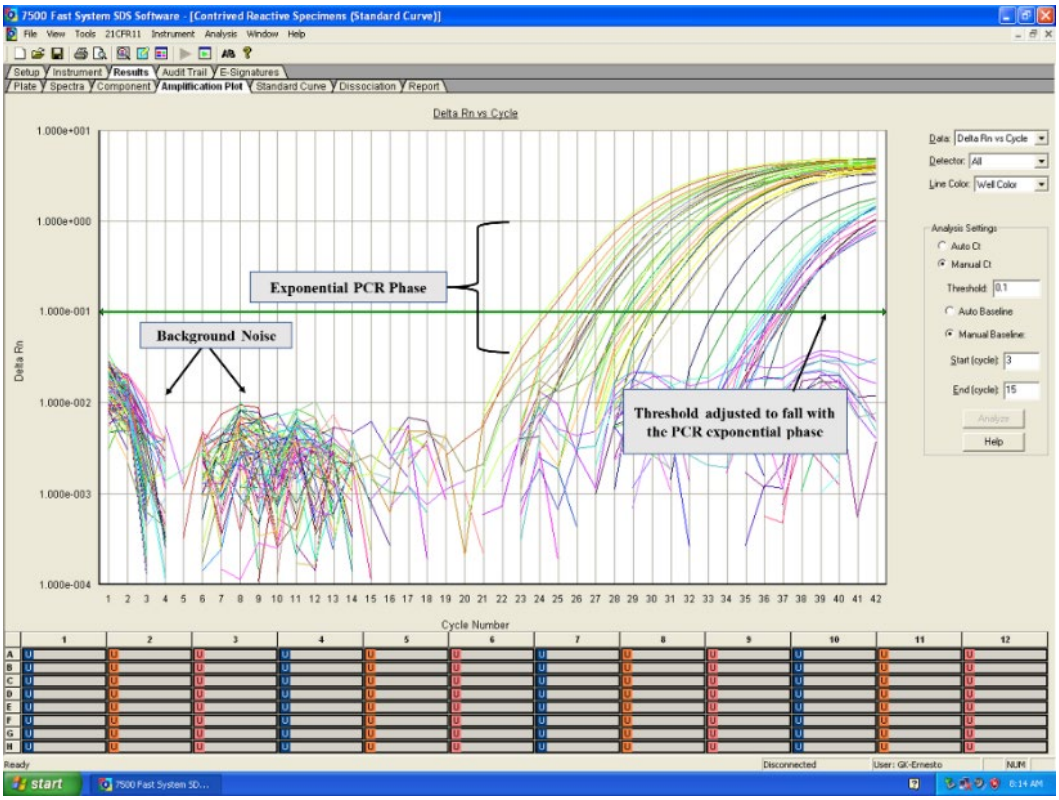
Annealing and extension			Yes	58	01:00

- Program the instrument to record and store the Ct values starting at step 4, which is the first cycle of the PCR amplification.
- When all controls and samples are added, close the plate and start the run.

10.8 Analysis of Results

After completing the execution, save and analyze the data following the instructions of the instrument manufacturer. Analyses should be performed separately for each target using a manual threshold setting. Thresholds should be adjusted to fall within the exponential phase of fluorescence curves and above any background signal (see Figure 3). The procedure chosen to set the threshold should be used consistently.

Figure 3. Amplification diagram



10.9 Interpretation of the Results

Interpretation of Controls

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the assay may have been set up and/or executed improperly, or reagents or equipment malfunction may have occurred and the patient's results cannot be interpreted. Control results should be interpreted according to the criteria outlined in the below table.

Table 5. Expected Results for Test Controls

Control Type	External Control Name	Used to Monitor	N1	N2	RP	Expected Ct values
Negative	GK SARS-CoV-2 Negative Control	Reagent and/or environmental contamination	-	-	-	Undetermined
Positive	GK SARS-CoV-2 Positive Control	Substantial reagent failure including primer and probe integrity. Potential contamination during extraction	+	+	+	< 40.00 Ct
HSC (optional)	Human Specimen Control	Extraction Control	-	-	+	< 40.00 Ct

The GK SARS-CoV-2 Negative Control consists of nuclease-free water in the Real-Time RT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line.

- If a false positive occurs with one or more of the GK SARS-CoV-2 Negative Control reactions of the primer and probe, contamination of the sample may have occurred.
- Invalidate the run and repeat the test with stricter compliance with the procedure guidelines.

The GK SARS-CoV-2 Positive Control consists of a combination of DNA plasmids encoding the SARS-CoV2 N gene and RP target. The GK SARS-CoV-2 Positive Control should produce a positive result with an expected Ct value for each target included in the test of Ct<40.00.

- If the expected positive reactivity is not achieved, invalidate the entire sample run.
- Determine the cause of failed GK SARS-CoV-2 Positive Control reactivity, implement corrective actions, and document results of the investigation and corrective actions.
- Then repeat the sample testing once the root cause is eliminated.

The Human Specimen Control (HSC) is optional and can be used as an external nucleic acid extraction control to demonstrate successful recovery of nucleic acid as well as extraction reagent integrity. If the HSC is included it should yield a positive result for the RNase P primer and probe set indicating the presence of sufficient nucleic acid from the human RNase P gene and that the sample is of acceptable quality. However, the HSC should be negative results for the N1 and N2 targets.

Patient Specimen Results Interpretation

If all controls are valid (see Table 5 above), patient results can be interpreted based on the amplification curve (or amplification plot) result from the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument 'Analyze' tab following the algorithm in Table 6 below.

Generally, clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.0 cycles (< 40.00 Ct), thus indicating the presence RNA for the human RNase P gene.

However, it is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. Sample results are interpreted as follows:

- **negative**: if all SARS-CoV-2 Targets (N1, N2) cycle threshold growth curves do not cross the threshold line within 40.00 cycles (< 40.00 Ct) and the RNase P growth curve crosses the threshold line within 40.00 cycles (< 40.00 Ct).
- **positive**: if one of the SARS-CoV-2 Targets (N1 or N2) AND the RNase P the cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct), If both of the SARS-CoV-2 Targets (N1 or N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct) the RNase P may or may not be positive for a valid positive result.
- **presumptive positive**: if only for one of the SARS-CoV-2 Targets cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct) but the RNase P does not cross the threshold line within 40 cycles, the result is considered valid but presumptive positive for SARS-CoV-2.
- **invalid**: if the growth curves for both SARS-CoV-2 Targets (N1, N2) and the RNase P marker do not cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is invalid. In this case, the extracted RNA from the specimen should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the retested sample is also negative for all targets and RNase P, the result is reported as invalid and a new specimen should be collected from the patient.

Interpret the results according to the patient specimen result interpretation algorithm (Table 6). The table summarizes the expected results for the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT. If a laboratory obtains unexpected results for assay controls or if invalid results are obtained and cannot be resolved through the recommended re-testing, please contact GK Pharmaceuticals CMO for consultation and possible specimen referral.

Table 6. Interpretation of Patient Specimen Results

N1 Target	N2 Target	RP Target	Interpretation of the Results	Report	Action
+	+	+ or -	Positive Result for SARS-CoV-2	Detected. Positive for SARS-CoV-2	Report positive results to healthcare provider and appropiate public health authorities
Only 1 of the two targets are positive		+	Positive Result for SARS-CoV-2	Detected. Positive for SARS-CoV-2	Report positive results to healthcare provider and appropiate public health authorities
Only 1 of the two targets are positive		-	Presumptive Positive Result for SARS-CoV-2	Presumptive Positive for SARS-CoV-2	Repeat extraction and rRT-PCR. If still presumptive positive a new sample should be collectedand tested
-	-	+	Negative Result for SARS-CoV-2	Not detected	Report negative results to healthcare provider and appropriate public health

					authorities. Consider testing for other pathogens.
-	-	-	Invalid Result	Invalid Result	Repeat extraction and rRT-PCR. If the repeated result remains invalid, collecting a new specimen from the patient.

11. Assay Limitations

- Laboratory personnnel should be trained and familiar with testing procedures and interpretation of results before performing the test.
- The performance of GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT was established using nasopharyngeal swab samples. Anterior nasal swabs and mid-turbinate nasal swabs are also considered acceptable specimen types for use with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction kits have not been validated for use with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT.
- If the virus mutates in the RT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably.
- Cross-reactivity with MERS-CoV was observed.
- False Positive results may arise from the contamination during specimen handling or preparation, or between patient samples.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined.
- False Negative results may arise from:
  a. Improper sample collection
  b. Degradation of the viral RNA during shipping/storage
  c. The presence of RT-PCR inhibitors
  d. Mutation(s) in the sequence of SARS-CoV-2 virus
- A false negative result may occur if there is an inadequate number of organisms present in the sample due to improper collection, transport or handling.
- RNA viruses in particular show substantial genetic variability. Although efforts were made to design RT-PCR assays in preserved regions of the viral genomes, variability resulting in erroneous matches between primers and probes and target sequences can decrease the performance of the test and possible false negative results.

12. Conditions of Authorization for the Laboratories

The GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

To assist clinical laboratories using the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT, the relevant Conditions of Authorization are listed below.

1) Authorized laboratories[1] using the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

2) Authorized laboratories using the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT will use the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT are not permitted.

3) Authorized laboratories that receive the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT will notify the relevant public health authorities of their intent to run the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT prior to initiating testing.

4) Authorized laboratories using the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropiate.

5) Authorized laboratories will collect information on the performance of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUAReporting@fda.hhs.gov) and GK Pharmaceuticals Contract Manufacturing Operations LLC (https://www.gkcmo.com/sars-cov-2) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT of which they become aware.

6) All laboratory personnel using the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT in accordance with the authorized labeling.

7) GK Pharmaceuticals CMO LLC, its authorized distributor(s) and authorized laboratories using the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT will ensure that any records associated with this test are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories".

13. Assay Performance or Performance Characteristics

Limit of Detection (LoD) - Analytical Sensitivity

The LoD study determined the lowest detectable concentration (copies/µL) of the SARS-CoV-2 at which at least 95% of all (true positive) replicates test positive. The preliminary LoD for the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT was determined using quantified genomic viral RNA (SARS-Related Coronavirus 2, Isolate USA-WA1/2020 from BEI Resources Catalog No. NR-52286) that was serially diluted into pooled nasopharyngeal clinical matrix obtained from SARS-CoV-2 negative individuals. A total of three concentration levels (4, 2, 1 copies/µL) were tested. Three triplicates of each dilution were extracted and tested according the instruction for use.

To confirm the preliminary LoD, the analyte concentration of 2 copies/µL was selected for confirmation with 20 replicates (Table 7). The samples were independently extracted using the QIAamp Viral RNA Mini Kit (Catalog No. 52906 (manual) and then tested with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT on the Applied Biosystems™

7500 Fast Dx Real-Time PCR Instrument with SDS (software v1.4). Based on this study, the LoD was confirmed to be 2 copies/µL (Table 7).

Table 7. LoD of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT

Preliminary LoD											
Target Level Copies/µL	Valid tested replicates	Detected replicates per result interpretation	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive			Internal Control Positive		
			n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
4	3	3 (100%)	3	36.5	100%	3	36.0	100%	3	34.8	100%
2	3	3 (100%)	3	36.6	100%	3	37.9	100%	3	33.3	100%
1	3	2 (67%)	1	36.1	33%	2	37.5	67%	3	33.2	100%
Confirmatory LoD											
Target Level Copies/µL	Valid tested replicates	Detected replicates per result interpretation	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive			Internal Control Positive		
			n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
2	20	20 (100%)	17	36.8	85%	17	36.0	85%	20	33.2	100%

* Note: The missed N1 and N2 targets were observed in different replicates. Per result interpretation a sample is interpreted as positive based on any one target SARS-CoV-2 target being positive as long as the internal RNase P control target is positive. As such all replicates generated a final positive result even if some replicates were missing to amplify one of the targets.

Inclusivity (Analytical Reactivity)

The sequences for the N1, N2 primers/probes used in the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT is identical to the N1, N2 primers/probes sequences used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel. The CDC has granted right-of-reference to assay developers to utilize the data generated in their authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel EUA.

Due to the increased SARS-CoV-2 genomic information in publicly available databases, an in-silico inclusivity analysis was performed September 3, 2020, to determine verify inclusivity.

The in-silico study was performed by using NCBI Basic Alignment Search Tool (BLAST) on the NCBI website for Betacoronavirus database to identify the largest regions of homology. The analysis included 10,692 SARS-CoV-2 full length/reference sequences. The total number of sequences containing mismatches with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT primer and probe sets that were found in the study was 98 (57 for the N1 target and 41 for the N2 target).

Table 8. In Silico Inclusivity Analysis of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT

Primer / Probe	**N1 probe**	**N1 forward**	**N1 reverse**	**N2 probe**	**N2 forward**	**N2 reverse**	**Total**
Mismatch No.*	32	17	8	21	14	6	98

Mismatch frequency (%)**	0.30	0.16	0.07	0.19	0.13	0.06	0.92

* Number of full length/reference sequences in the study for a specific mismatch
** Percentage of the full length/reference sequences not matching per specific target

No sequences were found to have more than one mismatch in any N1 primer/probe region. Only one sequence (0.009%) was identified with two nucleotide mismatches in the N2 forward primer. In addition, only one full length/reference sequence was identified sharing mismatches in N2 primer/probe region. As previously indicated by the CDC for the CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel the risk of that individual mismatches result in a significant loss in reactivity causing a false negative result is extremely low due to the design of the primers and probes and the thermocycling conditions of the test; where the melting temperatures for the primers/probes > 60°C and the annealing temperature at 58°C can tolerate up to two mismatches.

Specificity (Cross-Reactivity)
The cross-reactivity of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT was evaluated using both wet testing and *in silico analysis*. The in-silico analysis for the assay's primers and probes was performed using the NCBI Basic Alignment Search Tool (BLAST) to identify the largest regions of homology between any of the primers and probes to the genomes indicated.

Reference full length sequences from High priority pathogens of the coronaviridae family as well as other high-profile pathogens potentially present in respiratory specimens and relevant for the differential diagnostic of SARS-CoV-2 were included.

Table 9. Results of organism analyzed *in silico*

No	Organism	No	Organism
1	SARS-Coronavirus	22	*Bordetella pertussis*
2	MERS-Coronavirus	23	*Mycobacterium tuberculosis*
3	Human coronavirus 229E	24	*Mycoplasma pneumoniae*
4	Human coronavirus OC43	25	*Pneumocystis jirovecii (PJP)*
5	Human coronavirus HKU1	26	*Candida albicans*
6	Human coronavirus NL63	27	*Pseudomonas aeruginosa*
7	Human Adenovirus 71	28	*Staphylococcus epidermis*
8	Human Metapneumovirus (hMPV)	29	*Neisseria meningitidis*
9	Parainfluenza virus 1	30	*Neisseria elongata*
10	Parainfluenza virus 2	31	*Staphylococcus aureus*
11	Parainfluenza virus 4a	32	*Streptococcus salivarius*
12	Parainfluenza virus 4b	33	*Corynebacterium diphtheria*
13	Influenza A	34	*Bacillus anthracis (Anthrax)*
14	Influenza B	35	*Moraxella catarrhalis*
15	Enterovirus	36	*Leptospira*
16	Human respiratory syncytial virus A	37	*Chlamydia psittaci*

17	Human Rhinovirus	38	*Coxiella burnetii (Q-Fever)*
18	*Chlamydia pneumoniae*	39	Human genomic DNA
19	*Haemophilus influenzae*	40	*Legionella pneumophila*
20	*Streptococcus pneumoniae*	41	Bat betacoronavirus
21	*Streptococcus pyogenes*	42	Bat SARS-like coronavirus

In silico analysis for possible cross reactivity with organisms listed in Table 9 was conducted and homology ≥ 80% was found only for SARS coronavirus and Bat SARS-like coronavirus. However, neither is currently a circulating pathogen clinically relevant for the current SARS-CoV-2 pandemic.

Similar to the authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel EUA, the in-silico analysis for the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Hence an amplicon is not expected to be generated. In addition, because both viruses are not currently circulating the homology is not clinically relevant in the context of microbial interference testing. No false positive or false negative results for the N1 target are therefore expected for the GK ACCU-RIGHT SARS-CoV-2 RT-PCR Kit due to the presence of any of the analyzed organisms.

Similarly, analysis of the forward primer of the N2 target showed high homology to Bat SARS-like coronaviruses. However, the reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora was observed. As reported previously for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, no false positive or false negative results are therefore expected for the N2 target of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR Kit due to presence of any of the analyzed organisms.

For wet testing, a panel of respiratory pathogens were tested with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT. Sixteen (16) inactivated organisms and two (2) in vitro transcribed RNA which are spiked into SARS-CoV2 negative transport medium at the concentrations indicated in Table 10 were extracted by QIAamp Viral RNA Mini Kit (Catalog No. 52906). Then, the samples tested according to the Instructions for Use of the kit and none of the pathogens produced any detectable reactivity with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT.

Table 10. Cross reactivity of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT (wet testing)

No	Strain	Origin	Concentration cp/mL	Result
1	***Coronavirus*** (Strain: 229E)	Zeptometrix panel	1.0×10^6	Negative
2	***Coronavirus*** (Strain: NL63)	Zeptometrix panel	1.13×10^5	Negative
3	***Coronavirus*** (Strain: OC43)	Zeptometrix panel	3.3×10^5	Negative
4	**Enterovirus B111** (2015 Isolate)	Zeptometrix panel	8.0×10^6	Negative
5	***Haemophilus influenzae*** **type b**; Eagan	Zeptometrix panel	1.5×10^9	Negative

6	**Influenza A H1N1pdm** (NY/02/09)	Zeptometrix panel	0.7 x 10^5	Negative
7	**Influenza B** (Colorado/06/17)	Zeptometrix panel	0.9 x 10^5	Negative
8	***Mycoplasma pneumoniae*** M129	Zeptometrix panel	2.1 x 10^6	Negative
9	**Parainfluenza Virus Type 1**	Zeptometrix panel	2.3 x 10^7	Negative
10	***Pseudomonas aeruginosa*** (clinical isolate)	Zeptometrix panel	5.6 x 10^9	Negative
11	**Respiratory Syncytial Virus Type A** (RSV-A) (Isolate: 2006 Isolate)	Zeptometrix panel	3.3 x 10^5	Negative
12	**Rhinovirus** (Isolate: 10/2014 Isolate #1)	Zeptometrix panel	1.1 x 10^5	Negative
13	***Streptococcus pneumoniae*** 19F; Z022	Zeptometrix panel	1.5 x 10^9	Negative
14	***Streptococcus pyogenes*** Z018	Zeptometrix panel	6.0 x 10^8	Negative
15	***Candida albicans*** Z006	Zeptometrix panel	4.2 x 10^8	Negative
16	***Streptococcus salivarius*** Z127	Zeptometrix panel	5.5 x 10^8	Negative
17	**SARS-coronavirus**	In vitro transcribed RNA	1.3 x 10^3	Negative
18	**MERS-coronavirus**	In vitro transcribed RNA	1.3 x 10^3	Negative

Based on these results and what has been reported in other EUA using a similar combination of primer/probes, it can be reasonably concluded that the N1 and N2 primers and probes will not amplify or detect any of the analyzed pathogenic sequences.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD.

Manual nucleic acid extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen) and amplification was carried out on the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4. The results are summarized in Table 11.

Table 11. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	1.8 x 10^3 NDU/mL	N/A
MERS-CoV		N/A	D

Legend: NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
D: Detected using heat-inactivated MERS-CoV in cell culture media

Clinical Evaluation of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT using clinical samples previously tested by an EUA RT-PCR authorized assay

A study was performed to evaluate the performance of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT using 90 individual natural clinical nasopharyngeal swab specimens (45 negatives and 45 positives) collected in Puerto Rico by a CLIA certified high complexity laboratory. Swabs were collected into 3 mL viral transport media and tested with an EUA authorized RT-PCR test.

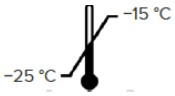
Leftover, deidentified samples were then tested with the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT in a randomized, blinded fashion according to the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT Instructions for Use and compared to the prior test result obtained with the EUA authorized test. Samples when tested with the comparator test ranged from Ct values of 13.6 to 38.5 for the N2 target. Out of 45 positive samples 10 samples (22%) are considered low positive by the comparator method (i.e., Ct> 35 for the N2 target of the comparator).

Performance of the GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT demonstrated 100% PPA and 100% NPA in comparison to the FDA authorized comparator assay. No false positive or false negative result was observed. The results are summarized in Table 12.

Table 12. Clinical evaluation with individual natural positives (confirmed) and negative nasopharyngeal swab samples

GK ACCU-RIGHT SARS-CoV-2 RT-PCR KIT	Molecular Comparator Method (CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel)		
	Positive	**Negative**	**Total**
Positive	45	0	45
Negative	0	45	45
Total	45	45	90
Positive Percent Agreement (PPA)	100% (95% CI: 92.1% – 100%)		
Negative Percent Agreement (NPA)	100% (95% CI: 92.1% – 100%)		

14. Symbols

Symbol	Indicates	Symbol	Indicates
	Medical device manufacturer		Biological risks
	Use-by date		Consult instructions for use
LOT	Manufacturer's batch code		Caution
REF	Manufacturer's catalog number		Indicates frozen temperature to which the *in vitro* diagnostic medical device can safely be exposed
SN	Manufacturer's serial number	IVD	*In vitro* diagnostic medical device
Rx Only	For Prescription Use only		Keep dry

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For technical support visit: https://www.gkcmo.com/sars-cov-2

References:

1. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use, accessible at https://www.fda.gov/media/134922/download