Procleix® SARS-CoV-2 Assay

For Use Under the Emergency Use Authorization (EUA) Only
For *In Vitro* Diagnostic Use

IVD

Rx Only
1000 Test Kit, 5000 Test Kit

CONTENTS

INTENDED USE

For *In Vitro* Diagnostic Use Under Emergency Use Authorization (EUA) Only.

The Procleix SARS-CoV-2 Assay is a qualitative *in vitro* nucleic acid test that uses transcription mediated amplification (TMA) for the qualitative detection of SARS-CoV-2 nucleic acid in anterior nasal and mid-turbinate nasal swabs, nasopharyngeal (NP) and oropharyngeal (OP) swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens obtained from individuals suspected of COVID-19 by their healthcare provider. Testing is limited 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.

Results are for the identification of SARS-CoV-2 RNA, which is generally detected in respiratory specimens during the acute phase of infection. Reactive sample results are indicative of the presence of SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Reactive sample 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.

Non-reactive sample results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Non-reactive sample results must be combined with clinical observations, patient history, and epidemiological information.

The Procleix SARS-CoV-2 Assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of transcription mediated amplification and hybridization protection assays as well as operation of the Procleix Panther System and *in vitro* diagnostic procedures. The Procleix SARS-CoV-2 Assay is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1] is a positive-sense single-stranded RNA virus that is a member of the family of *Coronaviridae* and the genus *Betacoronavirus*, which causes the coronavirus disease 2019 (COVID-19). Several members of the *Coronaviridae* family are known to cause respiratory infections, including the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) viruses. SARS-CoV-2 is a new virus that was first reported in an outbreak in Wuhan, Hubei province, China in December 2019 and spread globally in early 2020. Due to the widespread outbreak of the virus and severity of the disease, the World Health Organization (WHO) declared a pandemic of COVID-19 outbreak on March 11, 2020.[2]

COVID-19 symptoms include high fever, cough, shortness of breath and have ranged from asymptomatic to severe, including death. Older people and other people, regardless of age, with chronic conditions such as heart, lung diseases and diabetes, or with a compromised immune system seem to be at a higher risk of developing serious or fatal illness due to SARS-CoV-2 infection. Human-to-human transmission of SARS-CoV-2 occurs via respiratory droplets when an infected person coughs or sneezes and when a person touches contaminated surfaces and then touches their mouth, nose or eyes.[3]

PRINCIPLES OF THE PROCEDURE

The Procleix SARS-CoV-2 Assay uses the same transcription-mediated nucleic acid amplification (TMA) technology as other commercially available Procleix assays.

The Procleix SARS-CoV-2 Assay involves three main steps, which take place in a single tube: (1) sample preparation / target capture, (2) SARS-CoV-2 RNA target amplification by TMA, and (3) detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).

Specimens must be transferred to a secondary tube containing Procleix Specimen Extraction Buffer (SEB) prior to testing on the Procleix Panther System. The SEB lyses the cells and stabilizes nucleic acids. During sample preparation, RNA is isolated from specimens via target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Capture oligonucleotides that are homologous to highly conserved sequences of SARS-CoV-2 are hybridized to the SARS-CoV-2 RNA target, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube.

Target amplification occurs via TMA[5], which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target RNA sequence. The T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved by HPA[6] using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured by a luminometer and is reported as Relative Light Units (RLU).

Internal Control (IC) is added to each test specimen, control, and assay calibrator via the working Target Capture Reagent (wTCR). The IC in the Procleix SARS-CoV-2 Assay controls for specimen processing, amplification, and detection steps. IC signal is discriminated from the SARS-CoV-2 signal by the differential kinetics of light emission from probes with different labels. IC-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to SARS-CoV-2 is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA)[7] is a method used to differentiate between the signals from flasher and glower labels.

The Procleix SARS-CoV-2 Assay Calibrators are used to determine the assay cutoff and assess assay run validity in the Procleix SARS-CoV-2 Assay.

REAGENTS

Note: For ordering information, see MATERIALS PROVIDED and MATERIALS REQUIRED BUT AVAILABLE SEPARATELY sections

Procleix SARS-CoV-2 Assay Reagents

Internal Control Reagent

A HEPES buffered solution containing detergent and an RNA transcript.
*Store **unopened reagent** at –35° to –15°C.*

Target Capture Reagent

A HEPES buffered solution containing detergent, capture oligonucleotides, and magnetic microparticles. Internal Control Reagent must be added to Target Capture Reagent before use in the assay.

Store at 2° to 8°C (do not freeze).

Amplification Reagent

Primers, dNTPs, NTPs, and cofactors in TRIS buffered solution containing ProClin® 300 preservative.
*Store **unopened reagent** at –35° to –15°C.*

Enzyme Reagent

MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.
*Store **unopened reagent** at –35° to –15°C.*

Probe Reagent

Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.
*Store **unopened reagent** at –35° to –15°C.*

Selection Reagent

Borate buffered solution containing surfactant.
Store at 15° to 30°C.

Note: Do not use reagents or fluids past their labeled expiration date.

Procleix SARS-CoV-2 Assay Calibrators

C0

Procleix SARS-CoV-2 Assay Negative Calibrator
A HEPES buffered solution containing detergent.
Store at –35° to –15°C.

C1

Procleix SARS-CoV-2 Assay Positive Calibrator
A HEPES buffered solution containing detergent and a SARS-CoV-2 RNA transcript.
Store at –35° to –15°C.

Procleix Panther System Reagents

R1 — **Auto Detect 1**
Aqueous solution containing hydrogen peroxide and nitric acid.
Store at 15° to 30°C.

R2 — **Auto Detect 2**
1.6 N sodium hydroxide.
Store at 15° to 30°C.

W — **Wash Solution**
HEPES buffered solution.
Store at 15° to 30°C.

O — **Oil**
Silicone oil.
Store at 15° to 30°C.

DF — **Buffer for Deactivation Fluid**
Sodium bicarbonate buffered solution.
Store at 15° to 30°C.

Additional Reagent

Procleix Specimen Extraction Buffer

A HEPES buffered solution containing detergent.
Store at 18° to 25°C

STORAGE AND HANDLING INSTRUCTIONS

A. Room temperature is defined as 15° to 30°C.

B. ⚠ The Probe Reagent is light-sensitive. Protect this reagent from light during storage.

C. Do not use reagents or fluids after the expiration date.

D. Do not use assay-specific reagents from any other Procleix assay.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE TCR.

Note: If after removing the TCR from storage at 2° to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

F. Do not refreeze Internal Control, Amplification, Enzyme, and Probe Reagents after thawing.

G. Calibrators are single use vials and must be discarded after use. Do not refreeze calibrators after initial thaw.

H. If precipitate forms in the Wash Solution, Selection Reagent, Probe Reagent, Negative Calibrator, or Positive Calibrator, see instructions under REAGENT PREPARATION.

I. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness are indicative of microbial contamination), they should not be used.

J. Consult the following table for storage information.

Reagent/Fluid	Unopened Storage	Opened/Thawed Stability
Internal Control Reagent (IC)	–35° to –15°C	Prior to combining with TCR, 8 hours at RT
Target Capture Reagent (TCR)	2° to 8°C	20 hours at RT (RPI 250 File 3)*
working Target Capture Reagent (wTCR)		30 days at 2° to 8°C***; 82 hours at RT**
Amplification Reagent	–35° to –15°C	30 days at 2° to 8°C*** 20 hours at RT (RPI 250 File 3)*; 82 hours at RT**
Enzyme Reagent	–35° to –15°C	30 days at 2° to 8°C*** 20 hours at RT (RPI 250 File 3)*; 82 hours at RT**
Probe Reagent	–35° to –15°C	30 days at 2° to 8°C*** 20 hours at RT (RPI 250 File 3)*; 82 hours at RT**
Selection Reagent	RT	30 days at RT
Calibrators	–35° to –15°C	8 hours at RT
Auto Detect Reagents	RT	60 days at RT
Buffer for Deactivation Fluid	RT	60 days at RT
Oil	RT	60 days at RT
Wash Solution	RT	60 days at RT
Specimen Extraction Buffer	18° to 25°C	30 days at 18° to 25°C

RT = Room Temperature

*The 20 hours are only applicable to RPI 250 File 3 for preparation of unopened reagent bottles.

**The 82 hours must occur within 30 days which includes onboard stability. See the onboard stability table in REAGENT PREPARATION, section C.

*** ⚠ Reagents should be maintained at the appropriate storage condition when not in use. Unless reagents are in the RPI 250 or the Procleix Panther System, they should be returned to their appropriate storage conditions without delay.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Warning: Handle all specimens as if they are potentially infectious agents.

Note: Take care to avoid cross-contamination during the sample handling steps. For example, discard used material without passing over open tubes.

Note: All specimens should be handled as if infectious, using good laboratory procedures as indicated in the CDC "Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV".

Note: Change gloves often and if they come in contact with specimens.

Refer to CDC guidelines for sample collection and storage of anterior nasal and mid-turbinate nasal swabs, nasopharyngeal swabs (NP) and oropharyngeal swabs (OP), nasopharyngeal washes/aspirates or nasal washes/aspirates, and bronchoalveolar lavage specimens (BALs) at: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinicalspecimens.html.

A. Anterior nasal and mid-turbinate nasal, nasopharyngeal (NP), and oropharyngeal (OP) swab specimens collected in viral transport medium (VTM) recommended by CDC may be used. Nasopharyngeal washes/aspirates or nasal aspirates, and BALs can be collected in sterile containers such as the Cardinal Health 4 oz. sterile specimen container (Cat. No. 13594-130) or other appropriate sterile specimen containers without preservative media.

   The following types of flocked swabs and VTM/UTM were validated for use with the Procleix SARS-CoV-2 Assay:

   Copan FLOQSwabs (Cat. No. 503CS01)

   BD Universal Viral Transport Medium (Cat. No. 220220 or equivalent)

   Copan Universal Transport Medium (UTM) (Cat. No. 330C or equivalent)

   Similar VTM may be compatible but have not been validated. Handle specimens according to the manufacturer's specifications.

   Note: The following types of collection kits are NOT compatible for use:

   MicroCollect Virus Collection Swab Kit by CD Genomics

   Sample Collection Kit by BEAVER Biomedical Engineering Co., Ltd.

   PrimeStore Molecular Transport Medium (MTM) by Longhorn Vaccines and Diagnostics, LLC

   Swab Collection and Total Nucleic Acid Preservation System by Norgen Biotek

   Specimens containing guanidine thiocyanate or guanidine hydrochloride

B. Swab specimens must be vortexed for at least 5 seconds before processing and being transferred to a secondary tube containing Procleix Specimen Extraction Buffer (SEB) prior to testing on the Procleix Panther System.

   Note: When testing frozen specimens, allow specimens to reach room temperature prior to processing.

   Note: To reduce the risk of contamination, it is recommended to prepare an aliquot of SEB in a secondary container with enough volume for the specimens to be processed.

   Note: Always use a new pipette tip for each specimen.

   1. Clean work area and pipetters with diluted bleach (0.5–0.7% sodium hypochlorite).
   2. Prepare barcoded tubes using labels corresponding to the specimens to be tested.
   3. Fill each tube with at least 750 µL of SEB and cap, if not immediately used.
   4. Carefully open the specimen tube. Transfer an equal amount of the specimen to the tube containing SEB. Be careful not to transfer mucus, if present.

      Note: Volume may be adjusted, maintaining 1 part SEB and 1 part of specimen. Refer to the *Procleix Panther System Operator's Manual* for volume requirements based on tube dimension. The table below shows the recommended pipetting volumes based on tube diameter and number of test replicates. The total processing volume includes the required dead space volume as mentioned in the *Procleix Panther System Operator's Manual.* Consult Grifols Technical Service for guidance.

Tube Diameter	Number of Replicates	Minimum Specimen Volume (µL)	SEB Volume (µL)	Total Processing Volume (µL)
12–13 mm	1	750	750	1500
	2	900	900	1800
	3	1100	1100	2200
16 mm	1	900	900	1800
	2	1100	1100	2200
	3	1400	1400	2800

   5. Gently mix the resulting sample until homogeneous by pipetting up and down.

Note: Formation of bubbles in the samples must be avoided. If any samples contain bubbles, they must be carefully removed before loading on the Procleix Panther System using a Pasteur pipette, a pipette tip, or any other single-use method to avoid sample contamination.

6. Re-cap, or load directly onto the Procleix Panther System for testing.

C. Specimens combined with SEB (processed samples) may be stored at room temperature or at 2° to 8°C for 8 days.

D. Store original specimens according to the instructions of the collection kit manufacturer.

MATERIALS PROVIDED

Component	Part Number	Part Number
Procleix SARS-CoV-2 Assay Kit	**9052179** (1000 Test Kit)	**9052181** (5000 Test Kit)
Internal Control Reagent	4 x 2.8 mL	20 x 2.8 mL
Amplification Reagent	4 x 26 mL	20 x 26 mL
Enzyme Reagent	4 x 13.4 mL	20 x 13.4 mL
Probe Reagent	4 x 34.7 mL	20 x 34.7 mL
Target Capture Reagent	4 x 161 mL	20 x 161 mL
Selection Reagent	4 x 91 mL	20 x 91 mL
Procleix SARS-CoV-2 Assay Calibrators Kit	**9052180** (15 sets)	**9052182** (75 sets)
Negative Calibrator	15 x 2.2 mL	75 x 2.2 mL
Positive Calibrator	15 x 2.2 mL	75 x 2.2 mL

MATERIALS REQUIRED BUT AVAILABLE SEPARATELY

Procleix Assay Fluids Kit	**303344** (1000 Test kit)	
Wash Solution	1 x 2.9 L	
Oil	1 x 260 mL	
Buffer for Deactivation Fluid	1 x 1.4 L	
Procleix Auto Detect Reagents Kit	**303345** (1000 Test Kit)	
Auto Detect 1	1 x 245 mL	
Auto Detect 2	1 x 245 mL	
Procleix Specimen Extraction Buffer	**9052183**	**9052184**
Specimen Extraction Buffer	3 x 280 mL	14 x 280 mL
Disposables	**Quantity**	**Part Number**
(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)		
Multi-Tube Units (MTUs)	1 case of 100	104772
Waste Bag Kit	1 box of 10	902731
MTU Waste Cover	1 box of 10	504405
Reagent Spare Caps (TCR and Selection Reagents)	1 bag of 100	CL0039
Reagent Spare Caps (Amplification and Probe Reagents)	1 bag of 100	CL0042
Reagent Spare Caps (Enzyme Reagent)	1 bag of 100	501619
Equipment		
Procleix Panther System and operator's manual Procleix Reagent Preparation Incubator 250 (RPI 250) and operator's manual Independent Temperature Monitor (ITM)		
Other		
Advanced Cleaning Solution	1 bottle (255 mL)	PRD-04550

Note: Individual cataloged materials can be ordered separately as needed to meet individual site testing requirements.

OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH THE PROCLEIX SARS-COV-2 ASSAY

General Equipment/Software

For instrument specifics and ordering information, contact Grifols Technical Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Bleach (for use in final concentrations of 5 to 8.25% sodium hypochlorite and 0.5 to 0.7% sodium hypochlorite).

Alcohol (70% ethanol, 70% isopropyl alcohol solution, or 70% isopropyl alcohol wipes).

Disposable 1000 µL conductive filter tips (DiTis) in rack approved for use with the Procleix Panther System. Contact Grifols Technical Service for approved tips.

Tubes for processed samples, compatible with the Procleix Panther System. Refer to the *Procleix Panther System Operator's Manual* for volume requirements based on tube dimension.

PRECAUTIONS

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

B. For Rx use only.

C. This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests.

D. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

E. 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.

F. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

G. DO NOT test specimens that contain guanidine thiocyanate or guanidine hydrochloride, it reacts with sodium hypochlorite to generate a highly toxic gas.

H. When performing testing with different Procleix Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing. In addition, verify that the correct set of reagents is being used for the assay that is being run.

I. Specimens may be infectious. Use Universal Precautions when performing the assay. Proper handling and disposal methods should be established according to local, state, and federal regulations. Only personnel adequately qualified as proficient in the use of the Procleix SARS-CoV-2 Assay and trained in handling infectious materials should perform this procedure.

J. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

K. The Enzyme Reagent contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

L. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix SARS-CoV-2 Assay and the *Procleix Panther System Operator's Manual* prior to performing an assay run.

M. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes, and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry, and follow appropriate site procedures.

N. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations. Thoroughly clean and disinfect all work surfaces.

O. Use only specified disposables.

P. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

Q. Avoid microbial and nuclease contamination of reagents. Use of filtered, disposable pipette tips is required on the Procleix Panther System.

R. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE AND HANDLING INSTRUCTIONS and REAGENT PREPARATION for specific instructions.

S. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens.

T. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See REAGENT PREPARATION for specific instructions.

U. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagent or fluids. The Procleix Panther System verifies reagent levels.

V. Some reagents of this kit are labeled with risk and safety symbols and should be handled accordingly. Safety Data Sheets are accessible from the manufacturer's website.

Procleix Amplification Reagent
 *Glycerol 4.802 Weight-%* *Proclin 300 0.023 Weight-%* **WARNING** H317 - May cause an allergic skin reaction H412 - Harmful to aquatic life with long lasting effects P280 - Wear eye protection/face protection
Procleix Selection Reagent
 *Boric Acid 1–5 Weight-%* *SodiumHydroxide 0.1–1 Weight-%* **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation H332 - Harmful if inhaled H333 - May be harmful if inhaled
Procleix Auto Detect 2
 *SodiumHydroxide 6.04 Weight-%* **DANGER** H314 - Causes severe skin burns and eye damage P260 - Do not breathe dust/fume/gas/mist/vapors/spray P280 - Wear protective globes/protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor P280 - Wear eye protection/face protection
Procleix Buffer for Deactivation Fluid
 *SodiumHydroxide 1.12 Weight-%* *SodiumHypochlorite 0.49 Weight-%* **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation H411 - Toxic to aquatic life with long lasting effects

W. The Procleix Panther System groups a kit of reagents into a matched set the first time that it scans their barcodes during the inventory process and are required to be run as a set each subsequent time that they are loaded onto the Procleix Panther System. Bottles belonging to a matched set cannot be swapped with bottles in other matched sets of reagents. Refer to the *Procleix Panther System Operator's Manual* for more information.

X. Refer to additional precautions in the *Procleix Panther System Operator's Manual*.

Y. DO NOT heat the Probe Reagent above 35°C when using the RPI 250. Refer to the *Procleix RPI 250 Operator's Manual*.

Z. Each calibrator is designed to be run in triplicate, and excess material in each vial should be appropriately discarded.

REAGENT PREPARATION

A. Room temperature is defined as 15° to 30°C.

B. Choose a new or opened matched set of reagents. Do not use reagents that have been used outside the Procleix Panther System, as the instrument verifies reagent volumes.

C. Verify that the reagents have not exceeded the expiration date and/or storage stability times, including onboard stability.

The Procleix Panther System tracks the number of hours each reagent and fluid is loaded onboard the analyzer. The Procleix Panther System will not start pipetting specimens if reagents have expired or exceeded their onboard stability. Consult the following table for onboard stability information.

Reagent/Fluid	Onboard Stability*
wTCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, Selection Reagent	70 hours
Wash Solution, Oil, Buffer for Deactivation Fluid, Auto Detect Reagents	60 days

*The onboard time must occur within the room temperature times listed in the STORAGE AND HANDLING INSTRUCTIONS.

D. Remove a bottle of Selection Reagent from room temperature storage.

Note: The Selection Reagent must be at room temperature before use.

1. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, precipitate may form.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix RPI 250 Operator's Manual*. Do not use if precipitate or cloudiness persists.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
4. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

E. Refer to the *Procleix RPI 250 Operator's Manual* to prepare the following reagents using the RPI 250: TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent.

F. Precipitate could form in the Procleix SARS-CoV-2 Probe Reagent when stored at 2° to 8°C. To facilitate dissolution of precipitate, use the RPI 250 to thaw all probe reagents at an average temperature of 32° ± 2°C not to exceed 35°C. Refer to the *Procleix RPI 250 Operator's Manual*. Ensure that precipitates in all probe reagents are dissolved. Do not use if precipitate or cloudiness is present.

Note: If precipitate is still present after thawing, Probe Reagent can be incubated with RPI 250 File 3 (room temperature) to facilitate complete dissolution of precipitate. The Probe Reagent may also be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the water bath should not exceed 30°C. If thawing is conducted on the lab bench, probe reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate.

G. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall to between 2° and 15°C. Wash Solution may be warmed to facilitate dissolution of precipitate. Do not use the RPI 250 to warm the Wash Solution. Temperature should not exceed 30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

H. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present (refer to instructions below).

I. Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

J. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. Place TCR into the RPI 250, and refer to the *Procleix RPI 250 Operator's Manual* for instructions.
3. Thaw one vial of Internal Control (IC) Reagent up to 24 hours at 2° to 8°C or up to 8 hours at room temperature. Do not use the RPI 250 to thaw Internal Control Reagent.
4. Mix the Internal Control Reagent thoroughly by gentle manual inversion or mechanical inversion using a laboratory rocker.

Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the Internal Control Reagent at 25° to 30°C in a water bath. Periodically remove Internal Control Reagent from water bath to gently invert until gel is dissolved.

5. Unload TCR from the RPI 250 and warm the Internal Control Reagent to room temperature.
6. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR).
7. Record the date Internal Control Reagent was added, wTCR expiration date (date Internal Control Reagent was added plus 30 days), and lot number used (IC LOT), in the space indicated on the TCR bottle.
8. Retain the IC vial to scan the barcode label into the system.

K. Thaw calibrators at room temperature. **Do not use the RPI 250 to thaw Procleix SARS-CoV-2 Assay Calibrators.**

Note: These are single-use vials which must be thawed prior to each run.

1. Mix calibrators gently by inversion to avoid foaming.
2. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the calibrators at 25° to 30°C in a water bath. Periodically remove calibrators from water bath to gently invert until gel is dissolved.

L. Record the date Wash Solution, Oil, Buffer for Deactivation Fluid, Auto Detect 1, and Auto Detect 2 were first opened and loaded onto the Prodeix Panther System (OPEN DATE) in the space provided on the label.

PROCEDURAL NOTES

Note: Refer to the *Procleix Panther System Operator's Manual* for operating instructions.

Note: Procleix Auto Detect Reagents and Procleix Assay Fluids (Wash, Oil, Buffer for Deactivation Fluid) may be used with any master lot of Procleix Assay Reagents that are run on the Procleix Panther System.

A. Procleix SARS-CoV-2 Assay Calibrators are master lotted with the Procleix SARS-CoV-2 Assay. The operator must ensure that the Procleix SARS-CoV-2 Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the master lot barcode sheet enclosed with each shipment of Procleix SARS-CoV-2 Assay Calibrators.

B. Replace bottles in the Universal Fluids Drawer when notified by the system. Refer to the *Procleix Panther System Operator's Manual*.

C. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix SARS-CoV-2 Assay prior to performing an assay run. This package insert must be used with the *Procleix Panther System Operator's Manual*, *Procleix RPI 250 Operator's Manual,* and any applicable technical bulletins.

D. RUN SIZE

For the Procleix SARS-CoV-2 Assay, each worklist may contain up to 250 tests, including Procleix SARS-CoV-2 Assay Calibrators.

E. EQUIPMENT PREPARATION

See the *Procleix Panther System Operator's Manual*.

F. RUN CONFIGURATION

1. Each run must have a set of Procleix SARS-CoV-2 Assay Calibrators.
2. For the Procleix SARS-CoV-2 Assay, a set of calibrators consists of one vial each of Negative Calibrator and Positive Calibrator. The Negative and Positive Calibrators are run in triplicate.

G. WORK FLOW

1. Prepare reagent in clean area.
2. The sample loading area must be amplicon-free.

H. DECONTAMINATION

1. The extremely sensitive detection of analytes by this test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5 to 0.7% sodium hypochlorite in water (diluted bleach). Allow bleach to contact surfaces for at least 15 minutes, then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. Follow instructions provided in the *Procleix Panther System Operator's Manual* for instrument decontamination and maintenance procedures.

ASSAY PROCEDURE

Procleix SARS-CoV-2 Assay Calibrators are used with the corresponding master lot of the Procleix SARS-CoV-2 Assay. The operator must check to ensure that the Procleix SARS-CoV-2 Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the Procleix SARS-CoV-2 Assay master lot sheet in use.

Refer to the PRINCIPLES OF THE PROCEDURE, SPECIMEN COLLECTION, STORAGE, AND HANDLING, REAGENT PREPARATION, and PROCEDURAL NOTES sections for information on the specimen handling, reagent preparation, and automated workflow.

For equipment preparation, rack setup, and assay procedure information, see instructions in the *Procleix Panther System Operator's Manual*.

QUALITY CONTROL PROCEDURES

Internal Control

The Internal Control (IC) monitors for problems in target capture, amplification, or detection of the IC sequence, including deviations to the assay procedure as it is added to each reaction via the Target Capture Reagent. The IC signal is distinguished from the SARS-CoV-2 target signal by differential kinetics of light emission from the labeled probes used for each target. Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to SARS-CoV-2 is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.

Calibrators for the SARS-CoV-2 Assay

The results obtained from the negative and positive calibrators are used to determine the validity of the run and to establish the assay cutoffs for the Internal Control signal and the Analyte signal.

- Negative Calibrator

  The Negative Calibrator is used to determine run validity/assay and IC cutoffs and to monitor for cross-contamination.

- Positive Calibrator

  The SARS-CoV-2 Positive Calibrator is also used to determine the assay cut-off value and run validity.

ACCEPTANCE CRITERIA FOR THE PROCLEIX SARS-COV-2 ASSAY

A. Run validity:

A run (also identified as a worklist) is valid if the minimum number of calibrators meet their acceptance criteria and are valid.

Calibrators are positioned at the beginning of each assay run for the Procleix SARS-CoV-2 Assay. There are three replicates of the Negative Calibrator located in positions 1–3, and three replicates of the Positive Calibrators located in positions 4–6.

The results obtained from the calibrators are used to determine the validity of the run and to establish the assay cutoffs for the Internal Control signal and the Analyte signal. The upper and lower acceptance RLU limits of the calibrators in the software control for deviations in the test procedure that could compromise assay performance (e.g., operator- or instrument-induced contamination or incorrect volume of reagent to the assay tube.)

1. In a Procleix SARS-CoV-2 Assay run, at least four of the six calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and two of the three Positive Calibrator replicates must be valid.
2. Calibrator acceptance criteria are automatically verified by the Procleix Panther System Software. If less than the minimum number of calibrator replicates is valid, the Procleix Panther System Software will automatically invalidate the run.
3. In a valid run, cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower).
4. If a run is invalid, sample results are reported as Invalid and all specimens must be retested (specimen combined with the Procleix Specimen Extraction Buffer). If there is an insufficient volume remaining of the processed sample, a new processed sample can be made from the residual clinical specimen and re-tested.

B. Sample validity:

1. In a valid run, a sample result is valid if the IC signal is equal to or above the IC cutoff, with the following exceptions:
   a. Specimens with an analyte signal (glower signal) greater than the analyte cutoff are not invalidated even if the Internal Control (IC) signal is below the cutoff.
   b. In the Procleix SARS-CoV-2 Assay, specimens with RLU values outside of the software limits are invalidated by the software and their reactive status cannot be assessed. The software also automatically invalidates Positive Calibrators with RLU values outside of the software limits.
2. A sample may also be invalidated due to instrument and results processing errors. Refer to the *Procleix Panther System Operator's Manual* for details.
3. All individual specimen results that are Invalid in a valid run must be retested (specimen combined with the Procleix Specimen Extraction Buffer). If there is an insufficient volume remaining of the processed sample, a new processed sample can be made from the residual clinical specimen and re-tested.

INTERPRETATION OF RESULTS

All calculations for the cutoffs are determined by the Procleix Panther System Software for each assay using calibrator RLU values: one for the SARS-CoV-2 Signal (glower signal) termed the SARS-CoV-2 Cutoff and one for the Internal Control Signal (flasher signal) termed the Internal Control Cutoff. For each sample, glower signal RLU value and flasher signal RLU value are determined. The RLU value divided by the Cutoff is abbreviated as Signal/ Cutoff (S/CO) on the report.

If a specimen is Nonreactive, the Analyte S/CO must be < 1.00, the Internal Control Signal (Flasher RLU) must be greater than or equal to the Internal Control Cutoff, and the RLU values must be within the limits defined in the software. If a specimen is Reactive, the Analyte S/CO must be ≥ 1.00 and RLU values must be within software limits. The Internal Control is competitive in the presence of high SARS-CoV-2 RNA titer; its signal can be suppressed with Flasher RLUs below the Internal Control Cutoff but the final result remains Reactive.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria	Actions
Nonreactive (SARS-CoV-2 Not-Detected)	Analyte S/CO < 1.00 AND Flasher RLU ≥ Internal Control Cutoff AND RLU values within software limits	Report result to provider and appropriate public health authorities.
Reactive (SARS-CoV-2 Detected)	Analyte S/CO ≥ 1.00 AND RLU values within software limits	Report result to provider and appropriate public health authorities.*
Reactive (Presumptive)	Re-test the specimen in duplicate. Analyte S/CO ≥ 1.00 and ≤ 2 AND RLU values within software limits	If both re-test replicates are nonreactive, the specimen shall be considered nonreactive for SARS-CoV-2 RNA. If one or both re-test replicates are reactive (i.e., S/CO ≥ 1.00), the specimen shall be considered reactive for SARS-CoV-2 RNA. The final test result should be reported to the provider and appropriate public health authorities.
Invalid	RLU values outside software limits OR Analyte S/CO < 1.00 AND Flasher RLU < Internal Control Cutoff	Re-test the specimen using residual volume of the processed sample (specimen combined with Procleix Specimen Extraction Buffer) OR if there is insufficient volume remaining of the processed sample, a new processed sample can be made from the residual clinical specimen.

Analyte S/CO = SARS-CoV-2 Cutoff; Flasher RLU = Internal Control Signal; RLU = Relative Light Units

* Additional testing should be conducted when a reactive result is obtained with a S/CO ≥ 1 and ≤ 2.
It is recommended to re-test the specimen in duplicate and determine the status of the specimen based on the re-test results.

1. If both re-test replicates are nonreactive, the specimen shall be considered nonreactive for SARS-CoV-2 RNA.
2. If one or both re-test replicates are reactive (i.e., S/CO ≥ 1), the specimen shall be considered reactive for SARS-CoV-2 RNA.

Retesting of specimens following an invalid run does not require the collection of a new specimen. Residual volume of the processed sample (specimen combined with Procleix Specimen Extraction Buffer) may be used or, if there is insufficient volume remaining of the processed sample, a new processed sample can be made from the residual clinical specimen.

See Note in section B. 4. of SPECIMEN COLLECTION, STORAGE, AND HANDLING for details on volume required to process specimens.

LIMITATIONS OF THE PROCEDURE

A. This assay has been developed for use with the Procleix Panther System only.

B. Test results may be affected by improper specimen collection, storage, or specimen processing.

C. The following types of collection kits are NOT compatible for use: MicroCollect Virus Collection Swab Kit by CD Genomics, Sample Collection Kit by BEAVER Biomedical Engineering Co., Ltd., PrimeStore Molecular Transport Medium (MTM) by Longhorn Vaccines and Diagnostics LLC, Swab Collection and Total Nucleic Acid Preservation System by Norgen Biotek Corporation, and any kit containing guanidine thiocyanate or guanidine hydrochloride.

D. The performance of this test was assessed for nasopharyngeal swab specimens. Oropharyngeal swabs, nasal swabs (self-collected under supervision of, or collected by, a healthcare provider), mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and BALs are also considered acceptable to test; however, performance of the Procleix SARS-CoV-2 Assay has not been established with these specimen types.

E. Certain substances may interfere with the performance of the assay.

F. Cross-contamination of samples can cause false positive results.

G. Assays must be performed, and results interpreted, according to the procedures provided.

H. Deviations from these procedures, adverse shipping and/or storage conditions, or use of outdated reagents, if applicable, may produce unreliable results.

I. Failure to achieve expected results may be an indication of an invalid run. Possible sources of error include test kit deterioration, operator error, faulty performance of equipment, specimen deterioration, or contamination of reagents.

J. Though rare, mutations within the highly conserved regions of the viral genome targeted by the primers and/or probes in the Procleix SARS-CoV-2 Assay may result in failure to detect the virus.

K. The Procleix SARS-CoV-2 Assay is designed to detect SARS-CoV-2 RNA in respiratory specimens. SARS-CoV-2 RNA may persist in certain organs and tissues, as well as other body fluids, longer than it is detectable in respiratory specimens.

L. The clinical performance has not been established in 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.

CONDITIONS OF AUTHORIZATION FOR LABORATORIES

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

However, to assist clinical laboratories using the Procleix SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below.

A. Authorized laboratories* using the Procleix SARS-CoV-2 Assay must include with test result reports of the Procleix SARS-CoV-2 Assay, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using the Procleix SARS-CoV-2 Assay must perform the Procleix SARS-CoV-2 Assay as outlined in the Procleix SARS-CoV-2 Assay Instructions for Use. 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 perform the Procleix SARS-CoV-2 Assay are not permitted.

C. Authorized laboratories that receive the Procleix SARS-CoV-2 Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the Procleix SARS-CoV-2 Assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of the Procleix SARS-CoV-2 Assay and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Grifols Diagnostic Solutions Inc. (service.americas@grifols.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

F. All laboratory personnel using the Procleix SARS-CoV-2 Assay must be appropriately trained and use appropriate laboratory and personal protective equipment when handling this kit, and use the Procleix SARS-CoV-2 Assay in accordance with the authorized labeling.

G. Grifols Diagnostic Solutions Inc., its authorized distributor(s), and authorized laboratories using the Procleix SARS-CoV-2 Assay will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

* For ease of reference, this 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."

PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY OF THE PROCLEIX SARS-COV-2 ASSAY

Analytical sensitivity was evaluated by testing heat-inactivated virus SARS-CoV-2 NR-52286 from BEI Resources (Manassas, VA) and the AccuPlex SARS-CoV-2 Positive Reference Material from SeraCare (Milford, MA) diluted in Viral Transport Medium (VTM) and clinical specimen matrix at the indicated concentrations. One volume of each spiked sample was mixed with one volume of Procleix Specimen Extraction Buffer (SEB) prior to processing on the Procleix Panther System as per the Instructions of Use.

Three lots of reagents and three Procleix Panther Systems were used to test the indicated number of replicates of each copy level per reagent lot. Tables 1 and 2 demonstrate that the LoD with inactivated virus was similar to that obtained with the AccuPlex SARS-CoV-2 Positive Reference Material.

The limit of detection was further confirmed by spiking 60 and 90 copies/mL of the AccuPlex SARS-CoV-2 Positive Reference Material in clinical nasopharyngeal swab matrix that was tested in replicates of 20 with one reagent lot.

LoD of the Procleix SARS-CoV-2 Assay using Heat-Inactivated Virus

The Procleix SARS-CoV-2 Assay was 100% reactive at 60 copies/mL and above (Table 1).

Table 1. Detection of Heat-Inactivated Virus in VTM

Copies/mL	% Reactivity			
	Lot 1 (n = 20)	Lot 2* (n = 20)	Lot 3 (n = 20)	Combined** (n = 60)
200	100	100	100	100
60	100	100	100	100
20	95	65	90	83
6	60	30	65	52
2	20	10	20	17
0	0	0	0	0

* n = 34 for the negative panel
** n = 74 for the negative panel

LoD the Procleix SARS-CoV-2 Assay using the AccuPlex SARS-CoV-2 Reference Material

The Procleix SARS-CoV-2 Assay was at least 97% reactive at 60 copies/mL and 100% reactive at 200 copies/mL (Table 2).

Table 2. Detection of AccuPlex Reference Material in VTM

Copies/mL	% Reactivity			
	Lot 1* (n = 60)	Lot 2 (n = 60)	Lot 3 (n = 20)	Combined** (n = 140)
200	100	100	100	100
60	97	98	100	98
20	85	80	80	82
6	33	48	25	39
2	17	15	25	17
0	0	0	0	0

* n = 351 for the negative panel
** n = 431 for the negative panel

LoD of the Procleix SARS-CoV-2 Assay using the AccuPlex Reference Material in Clinical Specimens

To confirm the limit of detection in presence of the clinical matrix, negative nasopharyngeal swab specimens were tested in the absence or presence of 60 and 90 copies/mL of the AccuPlex Reference Material. The lowest target level at which more than 95% of 20 replicates for nasopharyngeal swab specimens produced positive results was 90 copies/mL.

Table 3. Detection of the AccuPlex Reference Material in Clinical Specimens

Copies/mL	# Specimens Tested	# Reactive	% Reactivity
0	38	0	0
60	20	18	90
90	20	20	100

INCLUSIVITY OF THE PROCLEIX SARS-COV-2 ASSAY

The inclusivity of the Procleix SARS-CoV-2 Assay was evaluated using *in silico* analysis of the assay capture oligonucleotides, amplification primers, and detection probes in relation to SARS-CoV-2 sequences available in the Global Initiative on Sharing All Influenza Data (GISAID) and the National Center for Biotechnology Information (NCBI) databases. The oligonucleotides used in the assay were compared to publicly available SARS-CoV-2 sequences as of January 2021.

Of the eight oligonucleotides present in the Procleix SARS-CoV-2 Assay, seven showed 100% identity with at least 97.893% of the sequences analyzed (90,888/92,844) and one had 100% identity with 65.470% (60,785/92,844) of the sequences. However, the Procleix SARS-CoV-2 Assay contains redundant primers for each oligonucleotide function. Therefore, for each function, 100% identity was observed with 98.844% (91,771/92,844) of 92,844 analyzed sequences (Table 4).

Table 4. Percent Identity of Each Oligo in the Procleix SARS-CoV-2 Assay to Publicly Available Sequences

Oligonucleotide	Number Sequences with 100% Identity/Total Sequence (%)	Number Sequences with 100% identity to at least one of the two oligos / Total Sequence (%)
Capture Oligonucleotide #1	92,711 / 92,844 (99.857%)	92,842 / 92,844 (99.998%)
Capture Oligonucleotide #2	91,234 / 92,844 (98.266%)	
Non-T7 Primer #1	91,748 / 92,844 (98.820%)	91,771 / 92,844 (98.844%)
Non-T7 Primer #2*	60,785 / 92,844 (65.470%)	
T7 Primer #1	92,739 / 92,844 (99.887%)	92,844 / 92,844 (100%)
T7 Primer #2	90,888 / 92,844 (97.893%)	
Probe #1	92,703 / 92,844 (99.848%)	92,703 / 92,844 (99.848%)
Probe #2	92,703 / 92,844 (99.848%)	

* mismatches at the 5' end of the primer; not expected to affect the performance of the assay due to the redundancy provided by Non-T7 Primer 1

In *silico* analysis of the assay capture oligonucleotides, amplification primers, and detection probes was performed in relation to SARS-CoV-2 B.1.1.7 variant sequences available in the GISAID database. For the capture oligos and probes, there are no mismatches to the B.1.1.7 variant sequences that were analyzed and at least one of the T7 primers and Non-T7 primers had 100% identity to their respective targets.

CROSS-REACTIVITY OF THE PROCLEIX SARS-COV-2 ASSAY

An *in silico* cross-reactivity analysis was conducted using the Basic Logic Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) database to identify sequence similarities to assay oligonucleotides to selected organisms/viruses shown in Table 5. The BLAST searches did not reveal any potential cross-reactivity except for with SARS coronavirus, which is in the same *Sarbecovirus* subgenus as SARS-CoV-2. When compared to the SARS coronavirus (NC_004718) genome sequence, there was 90% identity to NT7 primers, 90% identity to T7 primers, and 72% identity to the detection probes used in the Procleix SARS-CoV-2 Assay. Therefore, cross-reactivity with SARS coronavirus and other related *Sarbecovirus* was evaluated by laboratory wet testing (Table 6).

Table 5. Microorganisms Evaluated for Potential Cross-Reactivity by *In Silico* Analysis

High-Priority Pathogens From the Same Genetic Family	High-Priority Organisms/Viruses Likely in Circulating Areas
Human coronavirus 229E	Adenovirus
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1–4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius

Laboratory Wet Testing of Related *Sarbecovirus*

Genomic RNA purified from members of the *Sarbecovirus* genus was obtained from outside vendors, spiked in the Specimen Extraction Buffer solution, and tested using the Procleix SARS-CoV-2 Assay on the Procleix Panther System. The results are summarized in Table 6 below. No cross reactivity was observed in the preliminary wet testing.

Table 6. Evaluation of Potential Cross-Reactivity with Closely-Related Coronaviruses

Pathogen	Part Number	RNA Concentration Tested (copies/mL)	Result
Human coronavirus OC43	ATCC VR-1558DQ	$\geq 1 \times 10^5$	Nonreactive*
Human coronavirus HKU1	ATCC VR-3262SD	1×10^5 to 1×10^6	Nonreactive
Human coronavirus NL63	ATCC 3263SD	1×10^5 to 1×10^6	Nonreactive
Human coronavirus 229E	ATCC VR-740DQ	$\geq 1 \times 10^5$	Nonreactive
MERS-coronavirus RNA	ATCC VR-3248SD	1×10^5 to 1×10^6	Nonreactive
SARS-coronavirus RNA	EVAg 004N-02005	$\geq 1 \times 10^5$	Nonreactive

* Nonreactive = S/CO < 1.00

INTERFERING SUBSTANCES STUDY

An interfering substances study using endogenous and exogenous substances that could be associated with respiratory specimens was completed to assess the potential impact on performance of the Procleix SARS-CoV-2 Assay. Each endogenous/ exogenous interfering substance listed in Table 7 was evaluated at the highest medically relevant concentration (worst case) in the absence and presence of Accuplex SARS- CoV-2 positive reference material (spiked at ~1.33X LoD). Five replicates of each interfering substance alone and five replicates of each substance in the presence of SARS-CoV-2 RNA were tested with the Procleix Assay. No interference in the performance of the Procleix SARS-CoV-2 Assay was observed.

Table 7. Evaluation of Potentially Interfering Substances

Unspiked Specimens (Negative)										
Substance	Concentration*	n	#R	% R	IC-RLU		Analyte RLU		Analyte S/CO	
					Average	%CV	Average	%CV	Average	%CV
Control (VTM only)	N/A	5	0	0%	234058	2%	0	NA	0.00	NA
Mucin-High	2.50%	5	0	0%	231908	3%	2257	NA	0.01	NA
Mucin-Low	60 µg/mL	5	0	0%	230437	4%	54	NA	0.00	NA
Oxymetazoline	0.05%	5	0	0%	228621	2%	0	NA	0.00	NA
Beclomethasone	5 µg/mL	5	0	0%	223564	2%	0	NA	0.00	NA
Fluticasone	5 µg/mL	5	0	0%	226602	3%	114	NA	0.00	NA
Benzocaine	1.7 mg/mL	5	0	0%	229288	2%	0	NA	0.00	NA
Menthol	0.63 mg/mL	5	0	0%	224434	3%	931	NA	0.00	NA
Zanamivir	7.5 mg/mL	5	0	0%	226169	3%	0	NA	0.00	NA

Oseltamivir	25 mg/mL	5	0	0%	227378	3%	151	NA	0.00	NA
Mupirocin	10 mg/mL	5	0	0%	227107	2%	9867	NA	0.04	NA
Tobramycin	4 µg/mL	5	0	0%	226234	2%	721	NA	0.00	NA
Human Whole Blood	2% v/v	5	0	0%	225683	2%	11	NA	0.00	NA
Phenylephrine	0.50%	5	0	0%	227244	2%	0	NA	0.00	NA
Saline	15% v/v	5	0	0%	227813	2%	0	NA	0.00	NA
Zicam nasal gel	15% w/v	5	0	0%	235289	1%	0	NA	0.00	NA
Spiked Specimens (Positive)										
Substance	**Concentration***	**n**	**#R**	**% R**	**IC-RLU**		**Analyte RLU**		**Analyte S/CO**	
					Average	**%CV**	**Average**	**%CV**	**Average**	**%CV**
Control (VTM only)	N/A	5	5	100%	179596	17%	1058421	3%	4.39	3%
Mucin-High	2.50%	5	5	100%	165564	4%	1113368	1%	4.61	1%
Mucin-Low	60 µg/mL	5	5	100%	186225	3%	1031728	3%	4.28	2%
Oxymetazoline	0.05%	5	5	100%	204382	4%	1004660	7%	4.16	7%
Beclomethasone	5 µg/mL	5	5	100%	216754	11%	1027753	3%	4.26	3%
Fluticasone	5 µg/mL	5	5	100%	183031	7%	1047586	3%	4.34	3%
Benzocaine	1.7 mg/mL	5	5	100%	199932	21%	1038162	4%	4.30	4%
Menthol	0.63 mg/mL	5	5	100%	211194	21%	999153	5%	4.14	5%
Zanamivir	7.5 mg/mL	5	5	100%	164360	8%	1072852	3%	4.45	3%
Oseltamivir	25 mg/mL	5	5	100%	178702	13%	1033283	1%	4.28	1%
Mupirocin	10 mg/mL	5	5	100%	181672	10%	1051056	2%	4.36	2%
Tobramycin	4 µg/mL	5	5	100%	161164	9%	1081107	2%	4.48	2%
Human Whole Blood	2% v/v	5	5	100%	182427	4%	1034143	3%	4.29	3%
Phenylephrine	0.50%	5	5	100%	173731	11%	1069366	2%	4.43	2%
Saline	15% v/v	5	5	100%	182292	7%	1050226	4%	4.35	4%
Zicam nasal gel	15% w/v	5	5	100%	166691	14%	1038247	2%	4.30	2%

NA: Not Applicable
N =Number of Replicates, #R Number of Reactive Results, %R = Percent Reactivity
S/CO: Signal/Cut-off; S/CO ≥1.00 = "Reactive"; S/CO <1.00 = "Nonreactive"
%CV = Coefficient of Variation

CLINICAL PERFORMANCE OF THE PROCLEIX SARS-COV-2 ASSAY

For an evaluation of clinical sensitivity, 60 nasopharyngeal (NP) swab specimens from individuals suspected of COVID-19 were tested with the Procleix SARS-CoV-2 Assay. The 60 specimens, which were previously tested with an EUA-authorized RT-PCR assay, included 22 determined to be positive, 8 determined to be low positive based on the Ct values of the comparator assay, and 30 were SARS-CoV-2 negative samples. Specimens evaluated with the Procleix SARS-CoV-2 Assay were tested in a blinded fashion. Of the 30 positive specimens, all were correctly identified as reactive and of the 30 negative specimens, all were correctly identified as nonreactive by the Procleix SARS-CoV-2 Assay resulting in an NPA and PPA of 100% (Table 8).

Table 8. Percent Agreement of the Procleix SARS-CoV-2 Assay in NP Swab Specimens

Nasopharyngeal Swabs		**EUA-Authorized RT-PCR Comparator**		
		Positive	**Negative**	**Total**
Procleix SARS-CoV-2 Assay	**Positive (Reactive)**	30	0	30
	Negative (Nonreactive)	0	30	30
	Total	30	30	60
Positive Percent Agreement (PPA)		30/30; 100% (95% CI: 88.65–100.00%)*		
Negative Percent Agreement (NPA)		30/30; 100% (95% CI: 88.65–100.00%)*		

* Two-sided 95% score confidence intervals.

BIBLIOGRAPHY

1. **Coronaviridae Study Group of the International Committee on Taxonomy of Viruses.** The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. **5**: 536–544 (2020).
2. **World Health Organization.** Coronavirus disease (COVID-19) advice for the public. Retrieved 22 May 2020 from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public.
3. **Centers for Disease Control and Prevention.** Symptoms of Coronavirus. Retrieved 22 May 2020 from https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html
4. **Kacian D. L, T. J. Fultz.** 1995. Nucleic acid sequence amplification methods. U.S. Patent 5,399,491.
5. **Arnold L. J., P. W. Hammond, W. A. Wiese, N. C. Nelson**. 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem **35**: 1588–1594.
6. **Nelson N. C., A. Cheikh, E. Matsuba, M. Becker**. 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. **35**: 8429-8438.
7. **Centers for Disease Control and Prevention.** Research Use Only 2019 – Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Primers and Probes. Retrieved 22 May 2020 from https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html.

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Symbol Legend

Symbol	Symbol Meaning
	*In Vitro* Diagnostic Medical Device
Rx Only	For Prescription Use Only
	Manufacturer
	Caution
	Auto Detect 1
	Auto Detect 2
	Wash
	Oil
	Buffer for Deactivation Fluid
	Exclamation Mark - Irritant
	Corrosive
	Environmental Hazard

Procleix SARS-CoV-2 Assay

Rx only IVD

For use under EUA only

- This product has not been FDA cleared or approved, but 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.

Procleix SARS-CoV-2 Assay

By Grifols

GDSS-IFU-000059 v. 1.0 / 3059931

- 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.

The full labeling can be accessed at
grifols.force.com/DxNet

For a paper copy at no additional cost, please contact Grifols Technical Service Americas: (in U.S.) +1 (800) 452-6877 , or service.americas@grifols.com