BioGX
Molecular Made Easy

SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex

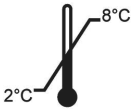
REF **400-058-E-HMP**

Σ 24/48 Reactions

Instructions For Use

For *In Vitro* Diagnostic Use

For use with BD MAX™ Open System Reagents on the BD MAX™ System, Bio-Rad CFX96 Touch™ and Applied Biosystems QuantStudio™ 5

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400-058-E-HMP BVPI Rev. 07-22SEP2023

PROPRIETARY NAME

BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex

INTENDED USE

BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex is a real-time reverse transcriptase, polymerase chain reaction (PCR) assay for use on the BD MAX™, Bio-Rad CFX96 Touch™ and Applied Biosystems QuantStudio™ 5 platforms for the qualitative detection of the presence of RNA from nucleocapsid phosphoprotein gene (N gene) of the SARS-CoV-2 coronavirus and the human RNase P gene. The primer and probe sets are based on the United States Centers for Disease Control and Prevention (US CDC) assay for specific detection of SARS-CoV-2 by amplifying two unique regions of the N gene (i.e., N1 and N2)[1,2,3]. Detection of the human RNase P gene serves as an endogenous Sample Processing Control (SPC). The following specimen types have been validated:

- Nasopharyngeal swab collection
  - Saline (0.85% NaCl), Copan Universal Transport Media (UTM®) and BD Universal Viral Transport (UVT)
- Oropharyngeal swab collection
  - Saline (0.85% NaCl), Copan Universal Transport Media (UTM®) and BD Universal Viral Transport (UVT)

The assay can be performed on the BD MAX™ automated nucleic acid extraction and real-time PCR instrument using the BD MAX™ ExK™ TNA-3 extraction strip and the accompanying BioGX UDP file. The real-time PCR assay can also be performed on the Bio-Rad CFX96 Touch™ and Applied Biosystems QuantStudio™ 5 using purified viral RNA extracted and purified using a validated magnetic bead or silica column nucleic acid extraction method.

The BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex reagent specifically targets the human RNase P gene to serve as an endogenous Sample Processing Control (SPC) RNA. This SPC serves as a control for the extraction of nucleic acids from the sample and as an internal amplification control. No external addition of SPC by the user is required.

The multiplex PCR assay is provided in BioGX Sample-Ready™ lyophilized format within a 2 mL screw cap tube. Each tube contains all PCR components such as primers, probes, enzymes, dNTPs, $MgCl_2$, and buffers required for real-time PCR-based testing of 24 BD MAX™ samples or 48 samples analyzed on the Bio-Rad CFX96 Touch™ or Applied Biosystems QuantStudio™ 5.

SUMMARY AND EXPLANATION

Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) is the novel betacoronavirus that causes the COVID-19 respiratory disease. The COVID-19 respiratory disease is transmitted amongst infected humans within populations via respiratory droplets from talking, coughing and sneezing[4,5]. Symptoms of COVID-19 range from mild illness (dry cough, fatigue, fever and shortness of breath) to severe illness and death[4].

COVID-19 was first identified on December 31, 2019 amongst patients suffering from pneumonia in Wuhan, China[4,5]. SARS-CoV-2 is the seventh coronavirus identified as a human pathogen that likely evolved within an animal host or within humans following zoonotic transfer[6].

As of April 11, 2020, the WHO has confirmed 1,610,909 SARS-CoV-2 infections and 99,960 SARS-CoV-2 deaths, globally[5]. The elderly, immunocompromised and those with cardiovascular disease, diabetes and chronic respiratory disease have been shown to be at higher risk for severe illness[4,5]. Ahead of global vaccination efforts, patient testing and social distancing measures mandated by world governments have proven to be the only way to stem transmission rates.

PRINCIPLES OF THE PROCEDURE

The BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex is to be used with either of the following platforms:

1. BD MAX™ Open System for automated patient sample processing and molecular analysis. The BD MAX™ System uses a combination of lytic and extraction reagents to perform cell lysis and nucleic acid extraction. Following enzymatic cell lysis at elevated temperature, the released nucleic acids are captured by magnetic affinity beads. To control for extraction efficiency, the BioGX reagents amplify and detect the human RNase P gene within each properly collected patient sample. The magnetic beads with bound nucleic acids are washed and the nucleic acids are eluted with heat in an elution buffer. The eluted nucleic acid is then mixed with the rehydrated Master Mix. The Master

Mix reagent and nucleic acid is then automatically dispensed into the BD MAX™ PCR Cartridge. Microvalves in the BD MAX™ PCR Cartridge are sealed by the system prior to initiating PCR to prevent evaporation and amplicon contamination.

2. Bio-Rad CFX96 Touch™ supports molecular analysis of nucleic acid extracted from a validated magnetic bead or silica column nucleic acid extraction method.

3. Applied Biosystems QuantStudio™ 5 supports molecular analysis of nucleic acid extracted from a validated magnetic bead or silica column nucleic acid extraction method.

The extracted template RNA is reverse transcribed into cDNA and target sequences are PCR amplified. The amplified target(s) are detected during amplification using hydrolysis probes labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. Probes labeled with different fluorophores are used to detect specific amplicons originating from SARS-CoV-2 Nucleocapsid Phosphoprotein gene (N1), SARS-CoV-2 Nucleocapsid Phosphoprotein gene (N2) and human RNase P gene (endogenous Sample Processing Control) in the following three different optical channels:

- Nucleocapsid Phosphoprotein gene (N2) detection:
  - FAM equivalent channel (495 nm / 520 nm)

- Nucleocapsid Phosphoprotein gene (N1) detection:
  - Texas Red equivalent channel (590 nm / 610 nm)

- RNase P endogenous (SPC) detection:
  - Cy 5.5 equivalent channel (690 nm / 705 nm)

When the hydrolysis probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of their specific target cDNA, the probes hybridize to their complementary sequences and are then hydrolyzed by the 5'-3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the cDNA template. As a result, the fluorophores are separated from their quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the three optical channels used for the BioGX SARS-CoV-2 HMP - N1, N2 and RNase P Multiplex is directly proportional to the quantity of the corresponding probe that is hydrolyzed, and therefore generally proportional to the amount of synthesized target. The BD MAX™ System, Bio-Rad CFX96 Touch™ and Applied Biosystems QuantStudio™ 5 measure these signals at the end of each amplification cycle in real time, and interpret the data to provide a qualitative result for

each of the above targets. A positive result for the detection of target RNA is indicated by the presence of a real-time PCR growth curve and an associated Ct (Cycle threshold) value.

REAGENTS

Qty	REF	Contents	Tests
1	400-058-HMP	**BioGX SARS-CoV-2 HMP N1, N2 & RNase P Multiplex** Sample-Ready™ lyophilized PCR Master Mix containing polymerase, reverse transcriptase, nucleotides, specific molecular primers and probes, and PCR buffers.	24/48 tests per pouch
1	800-031-E	**BioGX Rehydration Buffer E*** Reagent tube containing BioGX Rehydration Buffer for use with lyophilized PCR Master Mix rehydration.	24/48 tests per pouch

*BioGX Rehydration Buffer E is only for use with BD MAX platform. For Bio-Rad CFX96 Touch™ and Applied Biosystems QuantStudio™ 5, lyophilized reagents are rehydrated with molecular grade water.

NOTE: Safety Data Sheets (SDS) are available at www.biogx.com or by request.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX™ automated nucleic acid extraction and real-time PCR instrument, Applied Biosystems Quantstudio™ 5 or Bio-Rad CFX96 Touch™ real-time PCR instrument
- BD MAX™ ExK™ TNA-3 (BD catalog no. 442828).
  Extraction Kits include Sample Buffer Tubes (SBT), Septum Caps, Extraction Tubes, 0.3 mL conical tubes and Unitized Reagent Strips sufficient for 24 tests.
- BD MAX™ PCR Cartridges (BD catalog no. 437519).
- BioGX Synthetic RNA Control Template Kit (BioGX catalog no. 720-0678):
  - Nucleocapsid phosphoprotein gene region (N1) (BioGX catalog no. 720-0206).
  - Nucleocapsid phosphoprotein gene region (N2) (BioGX catalog no. 720-0207).
  - Human RNase P gene (BioGX catalog no. 720-0208).
- Sterile Swab Collection Device appropriate for nasopharyngeal/oropharyngeal swab collection and storage in Copan universal transport media (UTM®),BD universal viral transport (UVT) or saline (0.85%).
- Thermo Fisher 8-tube strip tubes (catalog no. 4316567).
- Thermo Fisher 8-tube strip optical caps (catalog no. AB-0866).
- Thermo Fisher 96-well plates (catalog no. A36924).
- Thermo Fisher 96-well plate seals (catalog no. AB-1170).
- Bio-Rad 8-tube strip tubes (catalog no. TLS0851).
- Bio-Rad 8-tube strip optical caps (catalog no. TCS0803).
- Bio-Rad 96-well plates (catalog no. HSP9655).

- Bio-Rad 96-well plate seals (catalog no. MSB1001).
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent.
- Disposable nitrile gloves.

WARNINGS AND PRECAUTIONS

- BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex can be performed on the BD MAX™ automated nucleic acid extraction and real-time PCR instrument using the BD MAX™ ExK™ TNA-3 extraction strip and the accompanying BioGX UDP file or on Applied Biosystems Quantstudio™ 5 or Bio-Rad CFX96 Touch™

- Treat all biological specimens, including used Extraction Kits and PCR Cartridges, as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29 and in Biosafety in Microbiological and Biomedical Laboratories[7,8].

- Performance characteristics of this test have been established only with the specimen types listed in “Intended Use” section. The performance of this assay with other specimen types or samples has not been validated.

- Do not use the reagents if the protective pouches are open or torn upon arrival.

- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-8 °C.

- Do not remove desiccant from the PCR Master Mix pouches.

- Do not use Master Mix if the desiccant is not present or is broken inside the Master Mix pouches.

- Do not use reagent tubes if the screw cap has been opened or damaged.

- Do not mix reagents from different pouches and/or kits and/or lots.

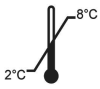
- Do not use expired reagents and/or materials.

- Each Master Mix and BioGX Rehydration Buffer tube is used to process 24/48 samples. Do not reuse Master Mix or BioGX Rehydration Buffer tubes.

- Refer to BD MAX™ ExK™ TNA-3 Extraction Kit Instructions for information about proper handling, cautions, and proper waste disposal.

- Do not mix septum caps between Sample Buffer Tubes or re-use septum caps as contamination may occur and compromise test results.
- Check BD Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kits are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Use clean gloves when handling extraction kit components and PCR reagents and buffer tubes.

STORAGE AND STABILITY



- BioGX recommends long-term storage of unopened pouches at 2-8°C. Refer to the product pouch label for shelf life duration.
- Reagents are stable at a temperature range of 2-30°C during shipment for 5 days.

- Reagents have been tested to demonstrate optimal performance when stored properly and consumed by the Expiration Date. Long-term stability studies are ongoing and the Expiration Date will be amended as additional data is available.

- Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting.
- Store unused rehydrated Master Mix for up to 24 hours at 2-8°C, protected from light.

- If the pouch is opened by the user, but the lyophilized reagent is not rehydrated, avoid exposure of lyophilized reagent to moisture and use the entire contents of the opened pouch within 2 months when stored at 2-8°C.

INSTRUCTIONS FOR USE

BD MAX™

(Operating software BD MAX™ Windows software V4.70A or later)

Install the BioGX Electronic User Defined Protocol on the BD MAX™

It will be necessary to import an Electronic User Defined Protocol (eUDP) onto the BD MAX™. The most current eUDP is available for download at www.biogx.com by using the drop down menu at the top right of the home page. Select "Education Center'' then select "Int. Product Documents''. Choose the appropriate product number under "Instructions for Use Manual & Product Inserts'' and download the eUDP. Please refer to the BD MAX™ user manual[9] for uploading instructions.

Bio-Rad CFX96 Touch™

(Operating software Bio-Rad CFX Maestro 1.1 or later)

Install the BioGX Protocol on the Bio-Rad CFX96 Touch™

It will be necessary to import the Bio-Rad CFX96 Touch™ Protocol and Plate file. The most current eUDP is available for download at www.biogx.com by using the drop down menu at the top right of the home page. Select "Education Center" then select "Int. Product Documents". Choose the appropriate product number under "Instructions for Use Manual & Product Inserts" and download the eUDP. Please refer to the Bio-Rad CFX96 Touch™ user manual[11] for uploading instructions.

Applied Biosystems QuantStudio™ 5

(Operating software QuantStudio Design & Analysis Software v1.5.0 or later)

Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5

It will be necessary to import the Applied Biosystems QuantStudio™ 5 template. The most current eUDP is available for download at www.biogx.com by using the drop down menu at the top right of the home page. Select "Education Center" then select "Int. Product Documents". Choose the appropriate product number under "Instructions for Use Manual & Product Inserts" and download the eUDP. Please refer to the Applied Biosystems QuantStudio™ 5 user manual[12] for uploading instructions.

Specimen Collection/Transport

Nasopharyngeal and oropharyngeal swab specimens should be collected, transported, and stored according to manufacturer recommendations, institutional and laboratory standard operating procedures.

Specimen Preparation

Nasopharyngeal/Oropharyngeal Swab in Copan UTM® or BD™ UVT Collection and Transport Devices (3 mL collection volume)

BD MAX™

Thoroughly vortex the sample prior to addition to the SBT. Pipette 500 µL of specimen into Sample Buffer Tube (SBT) and aseptically place a BD™ septum cap on the SBT. Pulse vortex the SBT for 1-3 seconds and load the SBT into the extraction tray.

Bio-Rad CFX96/Applied Biosystems QuantStudio™ 5

Pipette 500 µL of specimen into the appropriate sample extraction tube/plate and proceed with nucleic acid extraction using a validated magnetic bead or silica column extraction method or platform as per respective manufacturer's instructions for use.

Nasopharyngeal/Oropharyngeal Swab in Copan UTM® or BD™ UVT Collection and Transport Devices (1 mL collection volume)

BD MAX™

Pipette 150 µL of specimen and 350 µL molecular grade water into Sample Buffer Tube (SBT) and aseptically place a BD™ septum cap on the SBT. Pulse vortex the SBT for 1-3 seconds and load the SBT into the extraction tray.

Bio-Rad CFX96/Applied Biosystems QuantStudio™ 5

Pipette 150 µL of specimen and 350 µL molecular grade water into the appropriate sample extraction tube/plate and proceed with nucleic acid extraction using a validated magnetic bead or silica column extraction method or platform as per respective manufacturer's instructions for use.

Nasopharyngeal/Oropharyngeal Swab in 0.85% saline (3 mL collection volume)

BD MAX™

Thoroughly vortex the sample prior to addition to the SBT. Pipette 500 µL of specimen into Sample Buffer Tube (SBT) and aseptically place a BD™ septum cap on the SBT. Pulse vortex the SBT for 1-3 seconds and load the SBT into the extraction tray.

Bio-Rad CFX96/Applied Biosystems QuantStudio™ 5

Pipette 500 µL of specimen into the appropriate sample extraction tube/plate and proceed with nucleic acid extraction using a validated magnetic bead or silica column extraction method or platform as per respective manufacturer's instructions for use.

Nasopharyngeal/Oropharyngeal Swab in 0.85% Saline (1 mL collection volume)

BD MAX™

Pipette 150 µL of specimen and 350 µL molecular grade water into Sample Buffer Tube (SBT) and aseptically place a BD™ septum cap on the SBT. Pulse vortex the SBT for 1-3 seconds and load the SBT into the extraction tray.

Bio-Rad CFX96/Applied Biosystems QuantStudio™ 5

Pipette 150 µL of specimen and 350 µL molecular grade water into the appropriate sample extraction tube/plate and proceed with nucleic acid extraction using a validated magnetic bead or silica column extraction method or platform as per respective manufacturer's instructions for use.

Other Sample Types

This assay has been optimized for use with the specimen types and volumes described above. Use of any other specimen type, collection method, or sample volume may be inhibitory to the PCR or disrupt extraction without appropriate instrument setting Guardrail and processing volume adjustments. BioGX does not make claims for processing methods or sample types other than those described in this product insert.

Setting up the Unitized Reagent Strip on the BD MAX™

1. Wear nitrile gloves when handling Sample-Read™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Use only BD MAX™ ExK™ TNA-3 extraction kits with the BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex.
3. Load one extraction cartridge into the extraction tray per specimen to be tested.
4. Snap one BD MAX™ Extraction Tube into position 1 (Snap-1) of each Unitized Reagent Strip (Figure 1).

5. Snap one BD MAX™ empty 0.3 mL conical tube into position 3 (Snap-3) of each Unitized Reagent Strip (See Figure 1). Note: each BD MAX™ ExK™ TNA-3 extraction kit contains 24 empty 0.3 mL conical tubes.

6. Proceed with worklist generation and sample loading per BD MAX™ operating instructions. Select the appropriate User Defined Protocol (eUDP) provided by BioGX.

7. Using 1000 µL pipet tip, transfer **360 µL of BioGX Rehydration Buffer E** to one tube of Lyophilized BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex reagents.

8. Mix by gently pipetting up and down with 1000 µL pipet tip. (**IMPORTANT:** Keep the rehydrated Master Mix in a cold block or on ice until dispensed into the position 3-Snap BD MAX conical tube. Rehydrated Master Mix that is not used immediately can be stored up to 24 hours at 2-8°C, protected from light.)

9. Transfer **15 µL** of rehydrated Master Mix to the bottom of each empty 0.3 mL conical tube into position 3 (Snap-3) of each Unitized Reagent Strip (Figure 1).

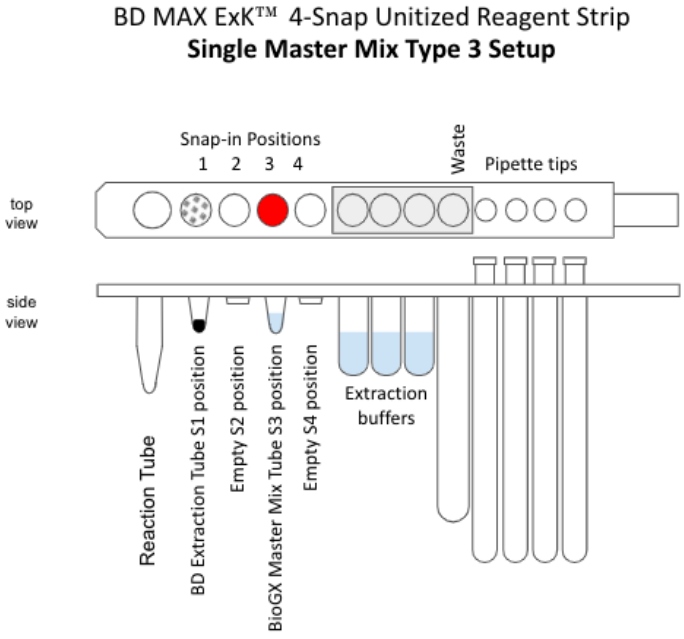


Figure 1. Diagram of BD MAX™ ExK™ TNA-3 4-snap Unitized Reagent Strip.

10. Lift the tray and briefly examine the bottom of each Unitized Reagent Strip to ensure all reagents are at the bottom of each tube.

11. Load the extraction tray and, if necessary, a new PCR card into the instrument, close the door, and click “Start Run”. Avoid unnecessary delay once racks are loaded.

Setting up 8-tube strips or 96-well plates for the Bio-Rad CFX96 Touch™ or Applied Biosystems QuantStudio™ 5

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1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube strips or 96-well plates.
3. Using 1000 µL pipet tip,transfer **500 µL of molecular grade water** (not included) to one tube of Lyophilized BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex reagents.
4. Mix by gently pipetting up and down with 1000 µL pipet tip. (**IMPORTANT**: Keep the rehydrated Master Mix in a cold block or on ice until dispensed into the tube/plate. If the rehydrated Master Mix cannot be used immediately, it can be stored up to 24 hours at 2-8°C, protected from light)
5. Transfer **10 µL** of rehydrated Master Mix to the bottom of each empty tube (8-tube strips) or empty (96-well plate).
6. To each well containing 10 µL of rehydrated Master Mix, add **5 µL** of patient sample extracted nucleic acid.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin to bring liquid to bottom.
9. Load 8-tube strips and/or 96-well plates into the real-time PCR platform and start. Avoid unnecessary delay once tubes/plates are loaded.

QUALITY CONTROL

CONTROL

Calibration of BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex is not required. Each BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex includes molecular primers and probes specific for the detection of the human RNase P gene to serve as an endogenous RNA sample processing control (SPC) present in a properly collected patient sample. No external addition of SPC is required. The SPC serves as both a sample extraction control and a PCR internal amplification control (IAC).

Laboratories must establish the number, type, and frequency of testing of control materials according to guidelines or requirements of local, provincial, state, and federal and/or country regulations or accreditation organizations in order to monitor the effectiveness of the entire analytical process. For general Quality Control guidance, the user may wish to refer to CLSI MM3 and EP12[7,8].

External Controls available from BioGX (catalog no. 720-0678) are treated as if they were patient samples. (Refer to the Table 1 in the Results Interpretation section for the interpretation of External Control assay results.)

It is recommended that one (1) External Positive Control and one (1) External Negative Control be run at least daily until adequate process validation is achieved on the BD MAX™ System in each laboratory setting. Reduced frequency of control testing should be in accordance with applicable regulations.

The External Positive Control is intended to monitor for substantial reagent failure. The External Negative Control is intended to detect reagent or environmental contamination (or carry-over) by target nucleic acids.

Various types of External Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program.

External Negative Control: A previously characterized sample known to be negative or SBT with 500 µL water added. BioGX recommends that the External Negative Control be prepared prior to the External Positive Control in order to reduce the potential for contamination as a result of control preparation.

External Positive Control: Commercially available control material from BioGX or other authorized control material may be used.

For the preparation of BioGX External Control suspensions, it is recommended that RNA suspensions be prepared in the Sample Buffer Tube (SBT). Please refer to BioGX SARS-CoV-2 & RNase P Synthetic RNA Control Templates (catalog number 720-0678) Please refer to BioGX Instructions for Use available for download at www.biogx.com by clicking on "Int. Product Documents" under "Education Center" and selecting the appropriate product under "Template Controls".

All External Controls should yield the expected results (positive for External Positive Control, negative for External Negative Control).

An External Negative Control that yields a positive result is indicative of sample handling and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.

An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the System Error Summary section of the BD

MAX™ System User's Manual[9] for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new assay kit.

RESULTS INTERPRETATION

BD MAX™

Results are available on the Results tab in the Results window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets the test result when the BioGX eUDP is used. Presence of one or more of the targets is possible, and will result in multiple targets being positive at once. Refer to the "Troubleshooting section of the BD MAX™ System User's Manual[9] for interpretation of warning and error codes.

Applied Biosystems QuantStudio™ 5

Results are available under the "Results" tab in the QuantStudio™ Design and Analysis Software. To download the data, the user can navigate to "Export" tab and export the data in a variety of formats (*.xls, *.xlsx, and *.txt). Alternatively, the user can navigate to "Print report" under "File" tab to preview and save the report in *.PDF format. Please refer to the QuantStudio™ 5 user manual for further instructions[12].

Bio-Rad CFX96 Touch™

Results are available to view and download in pdf format by navigating to the "Tools" tab and selecting the "Report" option on the Bio-Rad CFX Maestro™/Manager software in Data Analysis mode. Alternatively, the user can navigate to the "Export" tab and export all raw data sheets in a variety of formats (CSV, Test, Excel, XML). Please refer to the Bio-Rad CFX96 Touch™ user manual for further instructions[11].

External Negative and Positive Controls

If the positive or negative control does not exhibit the expected performance as described in the Control Interpretations Table 1, the assay may have been set up/or executed improperly, or reagent or equipment malfunction could have occurred. In this case, invalidate the run and re-test all samples in that run.

The RNase P gene serves as both a sample extraction control and an internal amplification control. In the event that both N1 and N2 region results are negative, an RNase P result must be positive for the result to be a valid negative result. When either N1 or N2 target result is positive, the RNase P result is ignored. If any of the above controls do not exhibit the expected performance as described, the assay may have been set up/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Table 1. Interpretation of BioGX external controls.

Control Type	Applicability for Monitoring	Expected Results		
		N1	N2	RNase P
Negative Control Addition of 500 µL water	Reagent and/or environmental contamination	-	-	-
Negative Control -Known Negative Sample		-	-	+
Extraction Control and RNase P Positive Control	Substantial reagent failure including primer and probe integrity	-	-	+
N1 and N2 Positive Control	Substantial reagent failure including primer and probe integrity	+	+	-

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the external positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The list of expected results are outlined in Table 2 and 3. If results are obtained that do not follow these guidelines, re-extract and re-test the sample. If repeat testing yields similar results, collect a fresh sample from the patient for testing.

Table 2. Interpretation of patient sample results[10].

N1 gene region	N2 gene region	RNase P	SARS-CoV-2 Interpretation	Action Recommended
+	+	+/-	POSITIVE	Report as POSITIVE
+	-	+/-	PRESUMPTIVE POSITIVE	if Positive upon Repeat, Report as POSITIVE[a]
-	+	+/-	PRESUMPTIVE POSITIVE	if Positive upon Repeat, Report as POSITIVE[a]
-	-	+	NEGATIVE	Report as NEGATIVE
-	-	-	UNRESOLVED[a]	Repeat Test[a]

[a]Repeat test by preparing a new extraction and PCR from the remaining patient sample collection to confirm a POSITIVE result (ie, confirmation of positive result with repeat test detecting N1 and/or N2). Conversely, repeat testing yielding neither N1 nor N2 detection confirms a NEGATIVE result.

REPEAT TEST PROCEDURE

In case of instrument failure, repeat testing can be performed by setting up a new run using the original sample/specimen and a fresh SBT as described above in the Specimen Preparation section.

LIMITATIONS OF THE PROCEDURE

- This device is not designed as the sole means of diagnosis of infectious disease. By the inherent nature of the technology used for nucleic acid extraction and detection, nucleic acid can be detected from dead organisms. The Intended Use is limited to the detection of the presence of the nucleic acid signature of an organism, and not the diagnosis of disease or disease state.
- This product is intended for use with specimens collected using specimen collection and transport devices listed in the "Equipment and Materials Required But Not Provided" section.
- This product should only be used with BD MAX™ Open System Reagents on the BD MAX™ System, Bio-Rad CFX96 Touch, Applied Biosystems QuantStudio 5.
- Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the package insert instructions and the User Manuals for the BD MAX™ System, Bio-Rad CFX96 Touch and Applied Biosystems QuantStudio 5 are necessary to avoid erroneous results.
- Good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
- A positive test result does not necessarily indicate the presence of viable infectious organisms. A positive result is indicative of the presence of target nucleic acid. A negative test result does not preclude the presence of infectious organisms and should not be used as the sole basis for treatment or other patient management decisions.
- As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection of the assay may be detected, but such results may not be reproducible.
- False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate cell lysis and/or extraction. The Sample Processing Control has been added to the test to aid in the identification of properly collected specimens, specimens that contain inhibitors to PCR amplification and as a control for reagent integrity and of the assay system as a whole.

- The BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex results may sometimes be Unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to instrument failure, and require retesting that can lead to a delay obtaining final results.
- Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown SARS-CoV-2 variants resulting in a false negative result with the BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex.
- The BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex requires the use of three (3) optical channels on the BD MAX™ System, Bio-Rad CFX96 Touch and Applied Biosystem Quantstudio 5 to detect fluorophores in the FAM, Texas Red and Cy 5.5 channels.

PERFORMANCE CHARACTERISTICS

Analytical and Diagnostic Specificity

Specificity was determined by analyzing negative sample matrix (Copan UTM nasopharyngeal specimens) spiked with positive control templates. The BioGX SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex was positive for all respective targets.

Analytical and Diagnostic Sensitivity

The analytical sensitivity for the SARS-CoV-2 HMP - N1, N2 & RNase P Multiplex was determined as follows: For BD MAX™ platform, contrived specimens (n=20) were generated by spiking known copy number of positive control synthetic SARS-CoV-2 N1 region RNA and SARS-CoV-2 N2 region RNA into negative Copan UTM nasopharyngeal swab collections. For Bio-Rad CFX96 Touch and Applied Biosystems QuantStudio 5 platforms, a dilution series of positive synthetic RNA samples for both N1 and N2 were added directly to rehydrated master mix (n=20). Analytical sensitivity (Limit of Detection, LoD) was defined as the lowest concentration at which 95% of all replicates tested positive. LoD has been confirmed to be equal or better with titered genomic viral RNA (Vircell Part number MBC137-R).

Table 3. LoD summary for BD MAX™

Target	LoD/mL (500 µL Sample input)	LoD/SBT (RNA copies per BD MAX SBT)	LoD/reaction (RNA copies per RT-PCR reaction)	Expected Cycle threshold (Ct)
N1 region	~1,000	508	30	32-34
N2 region	~1,000	508	30	32-34

Table 4. LoD summary for CFX96 Touch and QuantStudio 5

Target	LoD/mL (500 µL Sample input)[b]	LoD/reaction (RNA copies per RT-PCR reaction)	Expected Cycle threshold (Ct)
N1 region	600	30	33-35
N2 region	600	30	33-35

[b]Limit of detection based on 500 µL sample extraction with elution in 50 µL.

Inclusivity (*in silico*)

The BioGX SARS-CoV-2 N1 and N2 primers and probes are identical in sequence to those reported in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The CDC reported an *in silico* analysis of primer and probe sequences within their IFU (CDC-006-0019, Rev 02).[3] An alignment was performed with the oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 1, 2020 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel. All the alignments show 100% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one nucleotide mismatch with the N1 forward primer in one deposited sequence.

Cross-Reactivity (*in silico*)

Probe sequences for N1 and N2 used in the BioGX SARS-CoV-2 assay showed high sequence homology 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. Combining primers and probes, there are no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

Reproducibility

The reproducibility study detected synthetic RNA template analyzed independently by three different technicians using two BD MAX™ instruments, two Bio-Rad CFX96 Touch instruments and one Applied Biosystem QuantStudio 5 instrument over two separate days. All users obtained equivalent results on both instruments and on both days.

Manufacturing Reproducibility

Two independent lots were manufactured and were found to be equivalent based on internally established QC acceptance procedures.

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REVISION HISTORY

Revision	Date	Description of Change
07	22 SEP 2023	Clarification of long-term storage conditions and specify open pouch storage at 2-8C of reagents.
06	29 OCT 2021	Correction of manufacturing address on last page.
05	27 AUG 2021	Update pathway to direct to documents on BioGX website. Update results interpretation for Quantstudio™ 5 and CFX96 Touch™. Update symbol table, branding and shipment temperature.
04	25 JUN 2020	Update use of BioGX Rehydration Buffer E only for use with BD MAX, revise CFX96 and QuantStudio™ 5 rehydration protocol using molecular grade water, update of interpretation Table 2 and addition of FDA reference, genomic RNA equivalency confirmation.
03	16 APR 2020	Revise Analytical and Diagnostic Sensitivity text (page 14 & 15)
02	13 APR 2020	Update storage condition and setup of rehydrated Master Mix
01	11 APR 2020	Initial Release

SYMBOLS

Symbol	Meaning	Symbol	Meaning
	Catalog number		Contains sufficient for <n> tests
	CE mark of conformity		*In vitro* diagnostic medical device
	Do not reuse		Temperature limitation
	Batch code		Keep dry
	Caution		Keep away from sunlight
	Consult instructions for use		Expiration date
	Manufacturer		Biological Risks
	Control		

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