BioGX
Molecular Made Easy

COVID-19, Flu A, Flu B, RSV OSR for BD MAX™

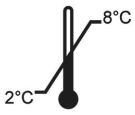
REF **400-060-G-MAX**

24 Reactions

Instructions For Use

For *In Vitro* Diagnostic Use

For use with BD MAX™ System





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PROPRIETARY NAME

COVID-19, Flu A, Flu B, RSV - RT-PCR for BD MAX™

INTENDED USE

BioGX COVID-19, Flu A, Flu B, RSV - RT-PCR for BD MAX™ is a real-time reverse transcriptase, polymerase chain reaction (PCR) assay for use on the BD MAX™ platform for the qualitative detection of the presence of RNA from SARS-CoV-2 (N1; nucleocapsid phosphoprotein gene[1,2,3]), Influenza A (matrix gene[4]), Influenza B (nonstructural gene[4]), RSV A/RSV B (nucleoprotein gene[4]) from the following specimens:

- **Nasopharyngeal swab collection**
  - -Copan Universal Transport Media (UTM®)
  - -BD™ Universal Viral Transport (UVT)
  - -Saline (0.85% NaCl)

- **Oropharyngeal swab collection**
  - -Copan Universal Transport Media (UTM®)
  - -BD™ Universal Viral Transport (UVT)
  - -Saline (0.85% NaCl)

The assay can only 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 BD MAX™ extraction reagent contains a Sample Processing Control (SPC) RNA, the presence of which is also detected by the BioGX multiplex assay. 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 a BioGX proprietary Sample-Ready™ lyophilized format sealed in a BD MAX™ tube. Each tube contains all PCR components such as primers, probes, enzymes, dNTPs, and buffers required for real-time PCR-based testing of one sample.

SUMMARY AND EXPLANATION

Influenza, also commonly known as the flu, is a contagious respiratory illness caused by the influenza virus. Influenza can cause mild to severe illness including, but not limited to, fever, cough, sore throat, muscle aches, and fatigue. The two major types of influenza virus, Types A and B, primarily infect humans. Influenza occurs globally with an annual attack rate estimated at 5%–10% in adults and 20%–30% in children[5]. Illnesses can result in hospitalization and death mainly among high-risk groups (the very young, elderly, or chronically ill). Worldwide, these annual epidemics are estimated to result in about 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths.

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections among young children in the United States and is the leading cause of death from respiratory illness in patients 65 years of age and older. RSV is estimated to be the second leading cause of lower respiratory infection deaths globally, resulting in 76,612 deaths in 2016[6]. Symptoms of RSV are similar to other respiratory infections, including Influenza. No specific treatment for RSV exists and researchers are working to develop vaccines and other antiviral treatment agents[7]. The two major antigenic subtypes, RSV A and RSV B, co-circulate during seasonal peaks of infection. Due to conflicting studies, the association of disease severity by either subtype is an area of continued investigation[8].

Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) is a novel betacoronavirus that causes the COVID-19 respiratory disease which is transmitted amongst infected humans via respiratory droplets. Symptoms of COVID-19 range from mild illness (dry cough, fatigue, fever, and shortness of breath) to severe illness and death[9]. Since the first cases of COVID-19 were identified in December 2019 in Wuhan, China, the disease has spread rapidly around the world. As of August 28, 2020, the WHO has confirmed 24,257,989 infections and 827,246 COVID-19 related deaths, globally[10]. Similar to Influenza and RSV, the elderly, immunocompromised, and those with cardiovascular disease, diabetes, and chronic respiratory disease have been shown to be at higher risk for severe illness. 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[11,12].

Recent clinical case studies have reported co-infection between SARS-CoV-2, Influenza, and RSV, and the incidence rate for a co-infection with SARS-CoV-2 and Flu A or SARS-CoV-2 and RSV might be as high as 65% and 10%, respectively, depending on geographical location and outbreak settings[13,14]. SARS-CoV-2, Influenza, and RSV share human-to-human transmission routes, seasonal occurrence, and overlapping clinical features. For an early etiological classification and a timely identification of possible co-infections, it is crucial to include several respiratory pathogens in the diagnostic algorithm. Especially during the winter months, hospital attendances and admission

rates for respiratory illnesses increase, impacting patient and bed management which drives the costs for isolation of suspected cases. The differentiation between common respiratory viruses such as Influenza, RSV and SARS-CoV-2 allows for effective infection prevention and control measures to be taken and helps clinicians to administer appropriate treatment agents to control the spread in the population successfully[15].

PRINCIPLES OF THE PROCEDURE

The BioGX COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ is to be used with the 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, an RNA Sample Processing Control is included in each BD MAX™ Extraction Tube. The 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 BioGX Rehydration Buffer, which is then transferred to the BioGX Sample-Ready™ lyophilized Master Mix tube in order to rehydrate the Sample-Ready™ lyophilized Master Mix. The rehydrated mix of amplification reagent and nucleic acid is then 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.

The extracted RNA is reverse transcribed into cDNA and target sequences are PCR amplified. The amplified target(s) is 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, Influenza A, Influenza B, RSV A and B, and the Sample Processing Control RNA in the assigned optical channels listed below:

- SARS-CoV-2 (N1) 475/520 channel
- Influenza A 530/565 channel
- RSV A/B 585/630 channel
- Influenza B 630/665 channel
- Sample Processing Control 680/715 channel

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 five optical channels used for the BioGX COVID-19, Flu A, Flu B, RSV 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 measures these signals at the end of each amplification cycle in real time and interprets 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	**400-060-MAX**	**BioGX COVID-19, Flu A, Flu B, RSV - RT-PCR for BD MAX™** Sample-Ready™ lyophilized PCR Master Mix containing polymerase, reverse transcriptase, nucleotides, specific molecular primers and probes, and PCR buffers.	24 tests per pouch
1	**800-033-G**	**BioGX Rehydration Buffer G** Reagent tube containing BioGX Rehydration Buffer for use with lyophilized PCR Master Mix rehydration.	24 tests per pouch

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
- 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 Lyophilized RNA Control Template Beads (1 x 10^5 copies/bead).
  - SARS-CoV-2 Nucleocapsid phosphoprotein gene (N1) (BioGX catalog no. 720-0206)
  - Flu A (BioGX catalog no. 720-0002)
  - Flu B (BioGX catalog no. 720-0003)
  - RSV A (BioGX catalog no. 720-0181)
  - RSV B (BioGX catalog no. 720-0182)
- 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% NaCl).
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent.

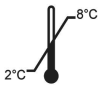
- Disposable nitrile gloves.

WARNINGS AND PRECAUTIONS

- BioGX COVID-19, Flu A, Flu B, RSV – OSR for BD MAX™ can only 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.
- 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[16] and in Biosafety in Microbiological and Biomedical Laboratories[17].
- 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 evaluated.
- 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 foil seal 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 a single sample. 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 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.
- Tightly reseal the pouch with unused reactions and immediately return to a refrigerator after opening.

- Avoid exposure to moisture and use the entire contents of the opened pouch within 2 months.

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™ System User's Manual[20] for uploading instructions.

Specimen Preparation

Copan UTM®, BD™ UVT and saline (0.85% NaCl) Collection and Transport Devices (3 mL collection volume)

BD MAX™
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.

Copan UTM®, BD™ UVT, and saline (0.85% NaCl) Collection and Transport Devices (1 mL collection volume)

BD MAX™
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. Note: For inhibitory samples containing excessive mucus, retest by pipetting 150 µL of specimen and 350 µL of molecular grade water into Sample Buffer Tube (SBT) and aseptically place a BD™ septum cap on the SBT.

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-Ready™ 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 COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ product.

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 BioGX Sample-Ready™ lyophilized PCR Master Mix reagent tube into position 2 (Snap-2) of each Unitized Reagent Strip. Check to make sure the Sample-Ready™ lyophilized cake is at the bottom of the tube prior to inserting into the Unitized Reagent Strip. The funnel-shaped cake may be in any orientation (v, >, ^, <) in the ***bottom*** of the tube.

6. Snap one BioGX Rehydration Buffer tube into position 3 (Snap-3) of each Unitized Reagent Strip. Check to make sure the buffer is at the bottom of the tube prior to inserting into the Unitized Reagent Strip.

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

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

9. Load the extraction tray and, if necessary, a new PCR card into the instrument, close the door, and click “Start Run”.

Note: Always first insert all Snap-1 tubes, then all Snap-2 tubes, then all Snap-3 tubes into the Unitized Reagent Strip. Snap position 4 will remain empty.

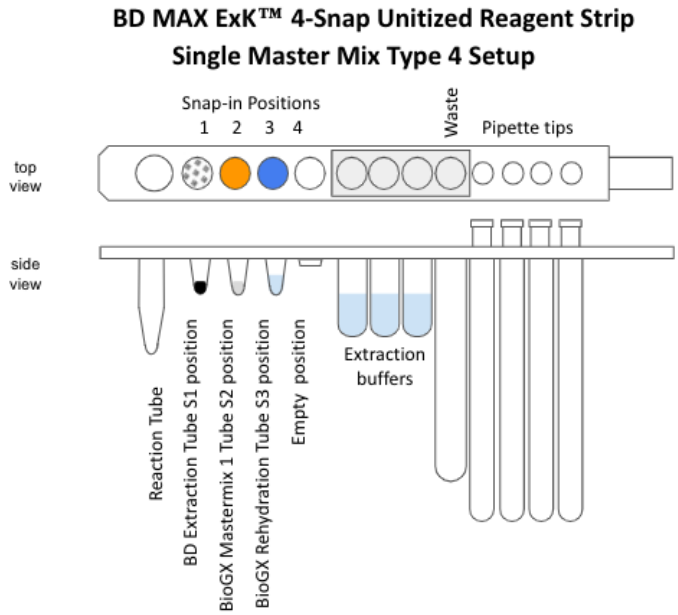


Figure 1. Diagram of BD MAX™ ExK™ 4-snap TNA-3 Unitized Reagent Strip.

QUALITY CONTROL

CONTROL

Calibration of BioGX COVID-19, Flu A, Flu B, RSV – OSR for BD MAX™ is not required. Each BioGX COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ includes molecular primers and probes specific for the detection of the exogenous sample processing control (SPC). 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[17,18]. External Controls available from BioGX are treated as if they were patient samples (Refer to 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 Negative Control is intended to detect reagent or environmental contamination (or carry-over) by target nucleic acids. Various types of External Controls are recommended including a previously characterized sample known to be negative or a No Template Control (NTC) to allow the user to select the most appropriate for their laboratory quality control program. BioGX recommends the NTC consist of molecular grade water to be added to the SBT. The same quantity of molecular grade water as sample volume that is being processed should be used. BioGX also recommends the External negative control be prepared prior to the External Positive Control in order to reduce the potential for cross-contamination during control preparation.

The External Positive Control is intended to monitor for substantial reagent failure. Commercially available control material from BioGX or other authorized sources may be used. For the BioGX External Control suspensions, it is recommended the RNA suspensions be prepared according to their respective IFU and then added to the Sample Buffer Tube (SBT). 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 outlined in Table 1. Briefly, positive results for External Positive Control, and negative for External Negative Controls. An External Negative Control yielding a positive result is indicative of environmental and/or sample cross-contamination. An External Positive Control that yields a negative result is indicative of a specimen handling or reagent preparation problem.

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[20] 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

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[20] for interpretation of warning and error codes.

External Negative and Positive Controls

If the positive or negative control does not exhibit the expected performance as described in 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 Sample Processing Control serves as sample extraction control and an internal amplification control. In the event that target results are negative, an SPC result must be positive for the viral target result to be identified as a valid negative result.

For further reference, please reference the product insert for Lyophilized Control Template Beads (BioGX Product Number Series 720-XXXX) which 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 "Template Controls".

Table 1. Interpretation of BioGX external controls.

Control Type	Applicability for Monitoring	Expected Results				
		Flu A	Flu B	RSV A/B	SARS CoV-2 (N1)	SPC
Negative Control -Addition of molecular grade water*	Reagent and/or environmental contamination	NEG	NEG	NEG	NEG	POS
Negative Control -Known Negative Sample		NEG	NEG	NEG	NEG	POS
SARS CoV-2 N1 Positive Control	Substantial reagent failure including primer and probe integrity	NEG	NEG	NEG	POS	POS
Flu A Positive Control	Substantial reagent failure including primer and probe integrity	POS	NEG	NEG	NEG	POS
Flu B Positive Control	Substantial reagent failure including primer and probe integrity	NEG	POS	NEG	NEG	POS
RSV A Positive Control	Substantial reagent failure including primer and probe integrity	NEG	NEG	POS	NEG	POS
RSV B Positive Control	Substantial reagent failure including primer and probe integrity	NEG	NEG	POS	NEG	POS

*BioGX recommends the NTC consist of molecular grade water to be added to the SBT. The same quantity of molecular grade water as sample volume that is being processed should be used.

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 is outlined in Table 2. 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[21].

SARS CoV-2 (N1)	Flu A	Flu B	RSV A/B	SPC	Result Interpretation
POS	**NEG**	**NEG**	**NEG**	**POS/NEG**	SARS CoV-2 POSITIVE
NEG	**POS**	**NEG**	**NEG**	**POS/NEG**	Influenza A POSITIVE
NEG	**NEG**	**POS**	**NEG**	**POS/NEG**	Influenza B POSITIVE
NEG	**NEG**	**NEG**	**POS**	**POS/NEG**	RSV A/B POSITIVE
NEG	**NEG**	**NEG**	**NEG**	**POS**	NEGATIVE
NEG	**NEG**	**NEG**	**NEG**	**NEG**	UNRESOLVED (UNR)[a]
Not Detected	**Not Detected**	**Not Detected**	**Not Detected**	**Not Detected**	INDETERMINANT (IND)[b]
Not Detected	**Not Detected**	**Not Detected**	**Not Detected**	**Not Detected**	INCOMPLETE (INC)[c]

[a]Repeat by preparing a new extraction and PCR from the remaining patient sample to confirm the result.
[b]Indeterminate due to BD MAX™ System failure. Refer to the "Troubleshooting" section of the BD MAX™ System User's Manual[20] for interpretation of warning and error codes.
[c]Incomplete run due to BD MAX™ System failure. Refer to the "Troubleshooting" section of the BD MAX™ System User's Manual[20] for interpretation of warning and error codes.

NOTE: In the presence of a high concentration positive result for the target, the SPC may be adversely affected (e.g., no amplification or delayed amplification).

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.
- 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 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/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 COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ 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, Flu A, Flu B, RSV A and RSV B variants

resulting in a false negative result with the BioGX COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™.

- The BioGX COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ requires the use of five (5) optical channels on the BD MAX™ System.

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 COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ was positive for all respective targets.

The BioGX COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ was run with ATCC MSA-1002 (20 Strain Even Mix Genomic Material) which does not contain genomic RNA for SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B. Results were negative for SARS-CoV-2, Flu A, Flu B, RSV A, and RSV B.

Analytical and Diagnostic Sensitivity

The analytical sensitivity for the BioGX COVID-19, Flu A, Flu B, RSV RT-PCR for BD MAX™ was determined with contrived specimens (n=20) generated by individually spiking quantified genomic viral RNA from: SARS-CoV-2 (Vircell Part No. MBC137-R), Flu A H1N1 (Vircell Part No. MBC028-R), Flu A H3N2 (Vircell Part No. MBC029-R), Flu B (Vircell Part No. MBC030-R), RSV A (Vircell Part No. MBC041-R), RSV B (Vircell Part No. MBC083-R) into negative Copan UTM®, and negative saline nasopharyngeal swab collections. Titered SARS-CoV-2 virus (Zeptometrix Part No. NATSARS(COV2)-ST) Analytical sensitivity (Limit of Detection, LoD) was defined as the lowest concentration at which 95% of all replicates tested positive (Table 3).

Table 3. LoD values for contrived Copan UTM®, and saline samples.

Target	Copan UTM® LoD (copies permL)	Expected Cycle Threshold (Ct)	Saline LoD (copies per mL)[d]	Expected Cycle Threshold (Ct)
SARS-CoV-2[e]	120	33-39	120	33-39
SARS-CoV-2[f]	120	33-36	120	33-37
Flu A (H1N1)[f]	960	32-39	320	34-39
Flu A (H3N2)[f]	160	32-36	160	30-33
Flu B[f]	80	30-33	80	32-37
RSV A[f]	80	29-32	80	29
RSV B[f]	2000	31-34	1200	33-37

[d]Inhibitory samples collections containing excessive mucus should be retested as described in the "Specimen Preparation" section of the IFU.
[e]Zeptometrix titered virus.
[f]Vircell quantified genomic RNA.

Inclusivity (SARS-CoV-2 *in silico*)

The BioGX SARS-CoV-2 N1 primers and probe 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 June 20, 2020, to demonstrate the predicted inclusivity of the CDC 2019 nCoV real-time RT-PCR Diagnostic panel. All the alignments show >99% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one sequence with a mismatch frequency of >1% in the N1 probe, all other sequences assessed had mismatch frequencies <1%. From the mismatch frequencies with <1%, two separate deposited sequences were observed to have two mismatches in the N1 probe and N1 reverse primer.

Cross-Reactivity (SARS-CoV-2 *in silico*)

Probe sequence for N1 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 templates analyzed independently by three different technicians using two BD MAX™ instruments 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.

REFERENCES

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REVISION HISTORY

Revision	Date	Description of Change
03	29 OCT 2021	Correction of manufacturing address on last page.
02	27 AUG 2021	Update pathway to direct to documents on BioGX website. Update symbol table, branding and shipment temperature.
01	24 SEP 2020	Initial Release

SYMBOLS

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

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