Manual

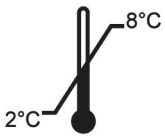
Flu A, Flu B, RSV A/B – OSR for BD MAX™

Version 01

REF 400-056-C-MAX

Σ 24 reactions

For *In Vitro* Diagnostic Use
For use with BD MAX™ Open System Reagents on the BD MAX™ System

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PROPRIETARY NAME

BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™

INTENDED USE

Flu A, Flu B, RSV A/B – OSR for BD MAX™ is a multiplex 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 Influenza A, Influenza B, RSV A and RSV B from the following specimens:

- **Nasopharyngeal swab collection**
  - -Copan Universal Transport Media (UTM®)
  - -BD™ Universal Viral Transport (UVT)
  - -Copan ESwab™
- **Pharyngeal swab collection**
  - -Copan Universal Transport Media (UTM®)
  - -BD™ Universal Viral Transport (UVT)
  - -Copan ESwab™
- **Nasal wash collection**

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, $MgCl_2$, 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. Gastrointestinal symptoms such as vomiting and diarrhea may also occur, but these symptoms are more common in children. The two major types of influenza

virus, Types A and B, primarily infect humans. While both can be responsible for seasonal flu epidemics, Influenza A is most commonly associated with seasonal flu.[1]

Influenza occurs globally with an annual attack rate estimated at 5%–10% in adults and 20%–30% in children. 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. In industrialized countries most deaths associated with influenza occur among people age 65 or older. Epidemics can result in high levels of worker/school absenteeism and productivity losses. Clinics and hospitals can be overwhelmed during peak illness periods. The precise effects of seasonal influenza epidemics in developing countries are not known, but research estimates indicate that a large percent of child deaths associated with influenza occur in developing countries every year.[2]

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. Symptoms of RSV are similar to other respiratory infections, including Influenza.[3]

PRINCIPLES OF THE PROCEDURE

The BioGX Flu A, Flu B, RSV A/B – OSR 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 RNA is reverse transcribed into cDNA and targets are PCR amplified. The amplified target 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 Influenza A, Influenza B, RSV A and B, and a Sample Processing Control in four different optical channels of the BD MAX™ System. Influenza A amplicons are detected in the 530/565 channel, RSV A and B amplicons are detected in the 585/630 channel, Influenza B amplicons are detected in the 630/665 channel, and the RNA Sample Processing Control is detected in the 680/715 channel.

When the 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 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 four optical channels used for the BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™ 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	400-056-MAX	**BioGX Flu A, Flu B, RSV A/B - OSR for BD MAX™** Sample-Ready™ lyophilized PCR Master Mix containing polymerase, reverse transcriptase, nucleotides, specific molecular primers and probes, Sample Processing Control-specific molecular primers and probe.	24 tests per pouch
1	800-028-C	**Rehydration Buffer Tube (C) Open System Reagents for BD MAX™** Reagent tube containing a rehydration buffer for use in Lyophilized PCR Master Mix rehydration.	24 tests per pouch

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

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX™ ExK™TNA-3 (BD catalog no. 442828)
  Extraction Kits include Sample Buffer Tubes (SBT), Septum Caps, Extraction Tubes, and Unitized Reagent Strips sufficient for 24 tests.
- BD MAX™ PCR Cartridges (BD catalog no. 437519)
- Appropriate sterile swab for nasopharyngeal swab collection and storage in viral transport media (Copan UTM® or BD™ UVT) or Liquid Amies media (Copan ESwab™).
- Appropropriate sterile collection device for nasal wash storage.
- Sterile Phosphate Buffered Saline (PBS)
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent.
- Disposable nitrile gloves.

WARNINGS AND PRECAUTIONS

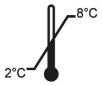
- Treat all biological specimens, including used Extraction Kits and PCR Cartridges, as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[4] and in Biosafety in Microbiological and biomedical Laboratories.[5]
- Performance 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 Rehydration Buffer tube is used to process a single sample. Do not reuse Master Mix or 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. Product is stable when shipped for under 5 days at ambient temperature.

- 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

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 on **www.biogx.com/eu** by clicking on “Product Documentation” and selecting the appropriate platform and product name. eUDPs can also be obtained by emailing BioGX at **eu@biogx.com**. Please refer to the BD MAX™ user manual for uploading instructions**.**

Specimen Collection/Transport

Swab and nasal wash specimens should be collected, transported, and stored according to institutional and laboratory standard operating procedures.

Specimen Preparation

Nasopharyngeal Swab (Copan ESwab™)
Pipette 50 µL of specimen and 450 µL PBS into a 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.

Pharyngeal Swab (Copan ESwab™)
Pipette 50 µL of specimen and 450 µL PBS into a 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.

Nasopharyngeal Swab (Copan UTM® or BD™ UVT)
Pipette 100 µL of specimen and 400 µL PBS into a 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.

Pharyngeal Swab (Copan UTM® or BD™ UVT)
Pipette 100 µL of specimen and 400 µL PBS into a 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.

Nasal Wash
Pipette 50 µL of nasal wash specimen and 700 µL of fresh viral transport media (Copan UTM® or BD™ UVT) into a 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:** Copan UTM® or BD™ UVT viral transport media support the necessary dilution of nasal wash specimens to achieve optimal extraction.

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 Flu A, Flu B, RSV A/B – OSR for BD MAX™. DO NOT use BD MAX™ mastermix or the blank 0.3 mL conical tubes from the BD MAX™ ExK™ TNA-3 extraction kit.
3. Load one extraction cartridge into the extraction tray per specimen to be tested.
4. Snap one BD MAX™ ExK™ TNA-3 Extraction Tube into position 1 (Snap-in 1) of each Unitized Reagent Strip. (See Figure 1)

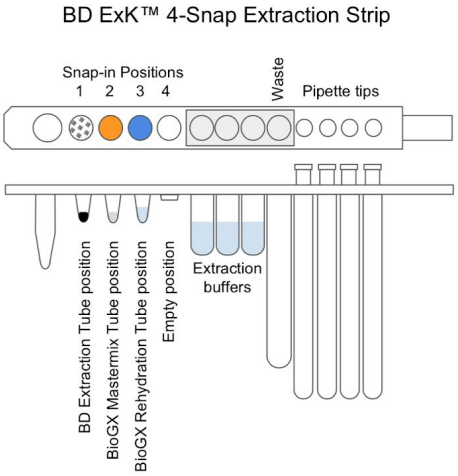


Figure 1 – Diagram of BD MAX™ ExK™ TNA-3 4-snap Unitized Reagent Strips

5. Snap one BioGX Sample-Ready™ lyophilized PCR Master Mix reagent tube into position 2 (Snap-in 2) of each Unitized Reagent Strip. Check to make sure the Sample-Ready™ lyophilized cake is at bottom of tube prior to inserting into 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-in 3) of each Unitized Reagent Strip. Check to make sure the buffer is at bottom of tube prior to inserting into 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-in position 4 will remain empty.

QUALITY CONTROL

CONTROL

Each BioGX Flu A, Flu B, RSV A/B - OSR for BD MAX™ includes molecular primers and probes specific for the detection of the RNA sample processing control (SPC) present in the BD MAX™ ExK™ TNA-3 Extraction Kit. No external addition of SPC is required. The SPC serves as both a sample extraction control and a PCR internal amplification control (IAC).

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. Possible results for each target are shown in Table 1. Presence of one or more of the targets is possible, and will result in multiple targets being positive at once.

Results*	Interpretation
Flu A POSITIVE	● The Influenza A target has a Ct within the valid range and endpoint above the minimum setting.
Flu B POSITIVE	● The Influenza B target has a Ct within the valid range and endpoint above the minimum setting.
RSV A/B POSITIVE	● The RSV A/B target has a Ct within the valid range and endpoint above the minimum setting.
Flu A NEGATIVE, **Flu B NEGATIVE,** OR **RSV A/B NEGATIVE**	● The respective target did not amplify and the SPC has a Ct within the valid range and endpoint above the minimum setting.
UNR	● Unresolved Result. No target amplification; No SPC amplification.

Results*	Interpretation
IND	• Indeterminate due to BD MAX™ System failure (with Warning or Error Codes**)
INC	• Incomplete Run (with Warning or Error Codes*)

*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.

**Refer to the "Troubleshooting section of the BD MAX™ System User's Manual for interpretation of warning and error codes.

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

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 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 BD MAX™ System User's Manual 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.
- As noted above, 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 specimens that contain inhibitors to PCR amplification and as a control for reagent integrity and of the assay system as a whole. The Sample Processing Control does not indicate if nucleic acid has been lost due to inadequate collection, transport or storage of specimens, or if cells have been adequately lysed.
- The BioGX Flu A, Flu B, RSV A/B – OSR 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 Influenza A, Influenza B, or RSV A/B resulting in a false negative result with the BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™.
- The BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™ requires the use of four (4) optical channels on the BD MAX™ System: 530/565 channel, 585/630 channel, 630/665 channel, and 680/715 channel.

PERFORMANCE CHARACTERISTICS

Analytical and Diagnostic Specificity

Specificity was determined by running the following negative specimens spiked with positive control template RNA:

-Nasopharyngeal specimens (Copan Eswab™ specimen collection)
-Pharyngeal specimens (Copan Eswab™ specimen collection)
-Nasopharyngeal specimens (Copan UTM® specimen collection)
-Pharyngeal specimens (Copan UTM® specimen collection)
-Nasal wash specimens

The BioGX Flu A, Flu B, RSV A/B - OSR for BD MAX™ yielded expected positive results for all respective template target spikes into negative specimens.

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

The QCMD 2017 Influenza virus A and B RNA (N=10) and the QCMD 2017 Respiratory Syncytial Virus RNA (N=10) were tested on the Flu A, Flu B, RSV A/B - OSR for BD MAX™ Assay in duplicate. Samples were spiked into BD MAX™ SBT tubes and subjected to full extraction mode utilizing BD MAX™ ExK™ TNA-3 Unitized Reagent Strips. All duplicate results were concordant and final results were in agreement with the expected target amplification.

QCMD 2017 Influenza virus A and B RNA Results

Target	Expected Result	Result
Influenza A (H1N1)	Influenza A	100% agreement
Influenza B (Victoria)	Influenza B	100% agreement
Influenza B (Victoria)	Influenza B	100% agreement
Influenza A and B Negative	Negative	100% agreement
Influenza A (H1N1)	Influenza A	100% agreement
Influenza A (H3N2)	Influenza A	100% agreement

QCMD 2017 Influenza virus A and B RNA Results (continued)

Target	Expected Result	Result
Influenza B (Yamagata)	Influenza B	100% agreement
Influenza A (H3N2)	Influenza A	100% agreement
Influenza A (H1N1 pdm09)	Influenza A	100% agreement
Influenza B (Victoria)	Influenza B	100% agreement

QCMD 2017 Respiratory Syncytial Virus RNA Results

Target	Expected Result	Result
RSV Type B	RSV A/B	100% agreement
RSV Type A	RSV A/B	100% agreement
Negative	Negative	100% agreement
RSV Type B	RSV A/B	100% agreement
RSV Type B	RSV A/B	100% agreement
RSV Type A	RSV A/B	100% agreement
RSV Type B	RSV A/B	100% agreement
RSV Type A	RSV A/B	100% agreement
RSV Type B	RSV A/B	100% agreement
RSV Type A	RSV A/B	100% agreement

Analytical and Diagnostic Sensitivity

The analytical sensitivity for the BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™ was determined as follows: A dilution series of positive synthetic RNA samples for each target were added to SBTs in duplicate. Analytical sensitivity (Limit of Detection, LoD) was defined as the lowest concentration at which 95% of all replicates tested positive.

Analytical sensitivity for BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™

Target	LoD (copies per SBT)
Flu A	6.35×10^2
Flu B	6.35×10^2
RSV A	6.35×10^2
RSV B	6.35×10^2

Analytical sensitivity during co-infection was tested by challenging the BioGX Flu A, Flu B, RSV A/B – OSR for BD MAX™ in pairs of high concentration (5,000X LOD) of one target against low concentration (5X LOD) of another for all possible pairs in the test. All low concentration targets tested positive and therefore were not outcompeted by amplification of the high concentration target.

Reproducibility

The reproducibility study detected synthetic target template analyzed independently by three different technicians using two BD MAX™ instruments. All users obtained equivalent results on both instruments.

Manufacturing Reproducibility

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

REFERENCES

1. Centers for Disease Control and Prevention. Influenza. http://www.cdc.gov. Accessed on August 30, 2016.
2. World Health Organization. Influenza Seasonal Fact Sheet No. 211, March 2014.
3. Centers for Disease Control and Prevention. Respiratory Syncytial Virus (RSV). http://www.cdc.gov. Accessed on August 30, 2016.
4. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
5. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Choosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.

REVISION HISTORY

Version	Date	Description of Change
01	09 SEP 2019	Initial Release

SYMBOLS

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Caution
	Consult instructions for use
	Manufacturer
	Contains sufficient for <n> tests
	Authorized Representative in the European Community
	Temperature limitation
	Keep dry
	Keep away from sunlight
	Expiration date
	Biological Risks
	Control

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