BioGX
Molecular Made Easy

# Mycobacterium tuberculosis Complex OSR for BD MAX™

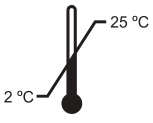
REF **400-010-W-MAX**

24 Reactions

# Instructions For Use

For *In Vitro* Diagnostic Use

For use with BD MAX™ System

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## PROPRIETARY NAME

BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™

## INTENDED USE

The BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™ is an automated *in vitro* diagnostic test reagent. The open system reagent (OSR) is used for the multiplex qualitative detection of DNA from Mycobacterium tuberculosis Complex (*IS6110* gene[1]), and a DNA sample processing control (SPC) from the following specimens from individuals at risk for presence of *Mycobacterium tuberculosis*:

- Pretreated sputum samples
- Bronchoalveolar Lavage (BAL) samples

The assay can only be performed on the BD MAX™ automated nucleic acid extraction and real-time PCR instrument using the BD MAX™ ExK™ DNA-1 Extraction Strip and the accompanying BioGX UDP file.

The BD MAX™ Extraction Reagent contains a Sample Processing Control (SPC) DNA, 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

Tuberculosis (TB) is caused by a bacterium called *Mycobacterium tuberculosis*. The bacteria usually attack the lungs, but *Mycobacterium tuberculosis* can attack any part of the body such as the kidney, spine, and brain. Not everyone infected with *Mycobacterium tuberculosis* becomes sick. As a result, two TB-related conditions exist: latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal.

Some people develop TB disease soon after becoming infected (within weeks) before their immune system can fight the TB bacteria. Other people may get sick years later, when their immune system becomes weak for another reason. Overall, about 5 to 10% of infected persons who do not receive treatment for latent TB infection will develop TB disease at some time in their lives. For persons whose immune systems are weak, especially those with HIV infection, the risk of developing TB disease is much higher than for persons with normal immune systems.

Sputum microscopy and microbiological culture is the 'gold standard' for the diagnosis of TB. However, microscopy has a low and variable sensitivity of detection and microbiological culture takes a significant amount of time to get a diagnosis. Direct nucleic acid amplification tests (NAAT) are playing a role in increasing the sensitivity of detection and decreasing the time to a diagnosis from weeks down to hours[1-4].

The BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™ is a real-time multiplex qualitative *in vitro* test reagent intended to be used by laboratory personnel trained in the use of the BD MAX™ automated real-time PCR system. The test is intended to aid in the diagnosis of *Mycobacterium tuberculosis* by detecting the presence of Mycobacterium tuberculosis Complex DNA extracted from Pretreated Sputum or Bronchoalveolar Lavage (BAL) collected from individuals at risk of infection.

## PRINCIPLES OF THE PROCEDURE

The BioGX Mycobacterium tuberculosis Complex – 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, a DNA Sample Processing Control is included in each BD MAX™ DNA Extraction Tube. The beads with bound nucleic acids are washed and the nucleic acids are eluted by 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 amplified DNA targets are detected 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 Mycobacterium tuberculosis Complex and a Sample Processing Control in two different optical channels of the BD MAX™ System:

- Mycobacterium tuberculosis 475/520 channel
- unused 530/565 channel
- unused 585/630 channel
- unused 630/665 channel
- Sample Processing Control 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 DNA template. As a result, the fluorophores are separated from their quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the two optical channels used for the BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™ is directly proportional to the quantity of the corresponding probe that is hydrolyzed, and therefore 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.

## REAGENTS

| Qty | REF | Contents | Tests |
|---|---|---|---|
| 1 | 400-010-MAX | **BioGX Mycobacterium tuberculosis Complex - OSR for BD MAX™**<br>Sample-Ready™ lyophilized PCR Master Mix containing polymerase, nucleotides, specific molecular primers and probes, Sample Processing Control-specific molecular primers and probe. | 24 tests per pouch |
| 1 | 800-029-W | **BioGX Rehydration Buffer Tube (W) Open System Reagents for BD MAX™**<br>Reagent tube containing BioGX 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™ automated nucleic acid extraction and real-time PCR instrument
- BD MAX™ ExK™ DNA-1 (BD catalog no. 442818).
  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).
- Sterile collection device appropriate for Sputum or BAL collection and storage.
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent.
- Disposable nitrile gloves.
- BioGX lyophilized Positive Control Template DNA Beads ($10^5$ copies/bead).
  - Mycobacterium tuberculosis BioGX part number 720-0017

## WARNINGS AND PRECAUTIONS

- BioGX Mycobacterium tuberculosis – 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™ DNA-1 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[5] and in Biosafety in Microbiological and Biomedical Laboratories[6].
- 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™ DNA-1 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 2-25°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 store the pouch in a dry location after opening.

- Avoid exposure to moisture and use the entire contents of the opened pouch within 2 months when stored at 2-8°C.

## 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 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[7] for uploading instructions.

### Specimen Collection/Transport

Sputum and BAL specimens should be collected, transported, and stored according to institutional and laboratory standard operating procedures.

### Specimen Preparation

**NOTE:** Appropriate locking-cap tubes or a lid-lock rack must be used when samples are boiled. The end user should use appropriate biosafety protocols (including a biosafety hood and respirator) when processing sputum samples that potentially contain *Mycobacteria.*

### Pretreatment of Sputum and BAL Samples

It is suggested that sputum and BAL samples be pretreated with a NALC-NaOH digestion/decontamination step followed by a proteinase K digestion and boiling treatment. For laboratories not currently processing sputum, the user may consider using the BD BBL® MycoPrep™ kit (BD catalog # 240862), a commercially available specimen decontamination kit for processing of Mycobacterial specimens.

Many variations of Proteinase K treatment may work. One strategy is to add 250 µL of Proteinase K solution (1 mg/mL) to the resuspended pellet obtained after NALC-NaOH treatment and neutralization, and incubate at 56°- 65°C for 30 minutes. After Proteinase K digestion, the sample should be heated to 100°C for 10-15 minutes (using an appropriate tube). **Once cooled, 200 µL of the specimen is added to the SBT, aseptically place the BD™ septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.**

For viscous samples the use of a mucolytic agent to reduce viscosity and support efficient DNA extraction is recommended. The use of BD BBL® MycoPrep™ kit (BD catalog # 240862) supports both mucolytic digestion and decontamination of natural flora.

Alternatively, Copan SL solution (Copan catalog #099CE.A) is a mucolytic agent that supports rapid digestion but does not provide decontamination of natural flora.

Manufacturer recommendations for BD BBL® MycoPrep™ and Copan SL solution should be followed. Depending on the mucopolysaccharide content of the specimen, the minimum recommended incubation time of 15 minutes to a maximum of 30 minutes for BD BBL® MycoPrep™ or a maximum of 120 minutes for Copan SL solution.

**Additional treatment for samples showing inhibition from previous analysis**

Add 40 µL of pretreated sputum/BAL to 160 µL water to dilute. Then add 25 µL of Proteinase K solution (1 mg/mL), briefly mix with a vortex mixer, and incubate at 60°C for 30 minutes. After Proteinase K digestion, the sample should be heated to 100°C for 30 minutes. Once cooled, 200 µL of specimen is added to SBT.

## Other Sample Types

⚠ This assay has been optimized for use with the sample types and volumes described above. Use of any other specimen type, collection method, or sample volumes may be inhibitory to the PCR or disrupt extraction without appropriate 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™ DNA-1 Extraction Kits with the BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™. DO NOT use BD MAX™ Master Mix or the blank 0.3 mL conical tubes from the BD MAX™ ExK™ DNA-1 Extraction Kit.

3. Load one extraction cartridge into the extraction tray per specimen to be tested.

4. Snap one BD MAX™ ExK™ DNA-1 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”. Snap-4 will remain empty.

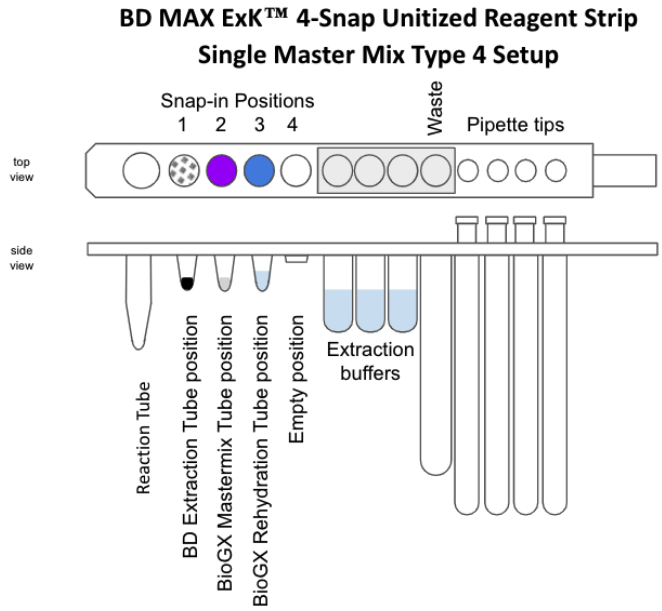


**Figure 1 – Diagram of BD MAX™ ExK™ 4-snap Unitized Reagent Strips**

**NOTE:** Always first insert all Snap-1 tubes, then all Snap-2 tubes, then all Snap-3 tubes into the Unitized Reagent Strip.

## QUALITY CONTROL

CONTROL

Calibration of BioGX Mycobacterium tuberculosis – OSR for BD MAX™ is not required. Each BioGX Mycobacterium tuberculosis Complex - OSR for BD MAX™ includes molecular primers and probes specific for the detection of the DNA sample processing control (SPC) present in the BD MAX™ ExK™ DNA-1 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).

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[6,8]. 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 DNA 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[7] 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. Possible results for each target for patient samples are shown in Table 2. Presence of one or more of the targets is possible and will result in multiple targets being positive at once.

### 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 | |
|---|---|---|---|
| | | MTB | SPC |
| Negative Control -Addition of molecular grade water* | Reagent and/or environmental contamination | NEG | POS |
| Negative Control -Known Negative Sample | | NEG | POS |
| MTB Positive Control | Substantial reagent failure including primer and probe integrity | POS | 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.**

| Results[a] | Interpretation |
|---|---|
| **MTB POSITIVE** | • The Mycobacterium tuberculosis Complex target has a Ct within the valid range and endpoint above the minimum setting. |
| **MTB NEGATIVE** | • The Mycobacterium tuberculosis Complex 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. |
| **IND** | • Indeterminate due to BD MAX™ System failure (with Warning or Error Codes[b]) |
| **INC** | • Incomplete Run (with Warning or Error Codes[b]) |

[a]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.

[b]Refer to the "Troubleshooting" section of the BD MAX™ System User's Manual[7] 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

- For *in vitro* diagnostic use.
- 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 Pretreated Sputum or BAL 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[7] 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 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 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 an 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 Mycobacterium tuberculosis Complex – 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 *Mycobacterium tuberculosis* resulting in a false negative result with the BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™.
- The BioGX Mycobacterium tuberculosis Complex – OSR for BD MAX™ requires the use of two (2) optical channels from the BD MAX™ System: 475/520 channel and 680/715 channel.

## PERFORMANCE CHARACTERISTICS

### Analytical Performance

The QCMD 2014 and 2015 *Mycobacterium tuberculosis* Panels (N=10 for both) were tested on the BioGX MTB Assay. Samples were spiked into BD MAX SBT tubes and subjected to full extraction mode utilizing BD MAX™ ExK™ DNA-1 Unitized Reagent Strips (Tables 3 and 4).

**Table 3. Analytical Performance QCMD 2014 *Mycobacterium tuberculosis* Results**

| Target | Result |
|---|---|
| Synthetic CSF MTB Positive (N=3) | 100% concordant (3/3) |
| Synthetic sputum MTB Positive (N=4) | 100% concordant (4/4) |
| Synthetic sputum M. xenopi (N=1) | 100% concordant (1/1) |
| Synthetic CSF Negative (N=1) | 100% concordant (1/1) |
| Synthetic sputum MTB Negative (N=1) | 100% concordant (1/1) |

**Table 4. Analytical Performance QCMD 2015 *Mycobacterium tuberculosis* Results**

| Target | Result |
|---|---|
| Synthetic CSF MTB Positive (N=4) | 100% concordant (4/4) |
| Synthetic sputum MTB Positive (N=3) | 100% concordant (3/3) |
| Synthetic sputum M. xenopi (N=1) | 100% concordant (1/1) |
| Synthetic CSF Negative (N=1) | 100% concordant (1/1) |
| Synthetic sputum MTB Negative (N=1) | 100% concordant (1/1) |

Clinical sputum samples were tested with the BioGX MTB Assay against the MTB ELITe MGB test on the Cepheid Smartcycler (Table 5).

**Table 5. BioGX vs. MTB ELITe MGB Kit on Cepheid Smartcycler (N=54)**

| Reference Result | Sample Size (N) | BioGX Result |
|---|---|---|
| Mycobacterium tuberculosis Complex Positive | N = 26 | 100% concordant (26/26) |
| Mycobacterium tuberculosis Complex Negative | N = 25 | 100% concordant (25/25) |
| Unresolved (by ELITe) | N = 3 | 100% concordant (3/3) |

**NOTE:** One MTB retrospective sample originally tested positive by the reference method, but tested negative by BioGX assay. When retested by the reference method the sample was negative. This data point was added to the Negative data set.

Clinical sputum samples and BAL samples were tested with the BioGX MTB Assay against culture as a reference (Tables 6 and 7).

**Table 6. Sputum Results**

| Reference Result | Sample Size (N) | BioGX Result |
|---|---|---|
| *Mycobacterium tuberculosis* Positive by Culture | N = 11 | 100% concordant (11/11) |
| *Mycobacterium tuberculosis* Negative by Culture | N = 7 | 100% concordant (7/7) |
| Unresolved by Culture | N = 6 | 3 Positive, 3 Negative by BioGX |

**Table 7. BAL Results**

| Reference Result | Sample Size (N) | BioGX Result |
|---|---|---|
| *Mycobacterium tuberculosis* Positive by Culture | N = 13 | 100% concordant (13/13) |
| *Mycobacterium tuberculosis* Negative by Culture | N = 14 | 86% concordant (12/14) – 2 positive by BioGX |

### Analytical Sensitivity

The analytical sensitivity for the BioGX Mycobacterium tuberculosis Complex- OSR for BD MAX™ Assay was determined as follows: Dilution series of quantified positive synthetic DNA samples (BioGX template controls) for each target and clinical (pretreated sputum sample) was determined for 20 independent contrived samples. Analytical sensitivity (Limit of Detection, LoD) was defined as the lowest concentration at which 95% of all replicates tested positive (Table 8).

**Table 8. Analytical sensitivity for BioGX Mycobacterium tuberculosis Complex– OSR for BD MAX™**

| Target | LoD (copies per mL) Pretreated sputum sample |
|---|---|
| Mycobacterium tuberculosis Complex | $1.90 \times 10^2$ |

*Assuming 100% extraction efficiency on the BD MAX™

### Analytical Inclusivity/Exclusivity

The BioGX MTB Assay primer set is designed to detect the Mycobacterium tuberculosis Complex IS6110 gene target. Amplicon search *in silico* analysis in BLAST indicates the primers will amplify and the probe will hybridize to all 284 Mycobacterium tuberculosis Complex organism submissions containing the IS6110 insert. This includes *Mycobacterium tuberculosis, Mycobacterium bovis* (BCG), *Mycobacterium microti, Mycobacterium africanum, Mycobacterium caprae, and Mycobacterium canettii.* Other than the sequences mentioned above, none of the other sequences in the BLAST (n) database (as of April 5, 2017) will amplify and report for the BioGX MTB Assay primer and probe set.

### Reproducibility

The reproducibility study was performed on pertussis toxin synthetic target template by three separate technicians independently on two BD MAX™ instruments. Using two lots of reagents, a series dilution of DNA template was run between 100,000X LoD and $10^{-1}$ LoD dilutions of the stock template. All samples from 1X LoD to 100,000X LoD were concordant positive between samples and technologists. All samples run at $10^{-1}$ LoD were concordant negative, as expected.

### Manufacturing Reproducibility

Seven independent lots were manufactured and were found to be equivalent based on internally established QC acceptance procedures. The lots included five production lots: #016-089-094, #016-145-163, #016-245-290, #016-267-325, #016-278-344 as well as two scale up production lots: #016-307-401 and #017-039-032.

## REFERENCES

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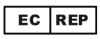
## REVISION HISTORY

| Revision | Date | Description of Change |
|---|---|---|
| 09 | 25 AUG 2025 | Update Manufacturing address from BioGX BV to BioGX Inc. in accordance with DTP0825. |
| 08 | 22 SEP 2023 | Clarification of long-term storage conditions and specify open pouch storage at 2-8°C of reagents. |
| 07 | 28 OCT 2021 | Correction of manufacturing address on last page. |
| 06 | 27 AUG 2021 | Updated reagent quantity per pouch; included BioGX positive control template part numbers and updated open pouch stability from one month to two months. Updated Snap-4 image, added external control processing and result interpretation. Updated unit of measure of LoD, symbol table, branding and shipment temperature. |
| 05 | 01 FEB 2019 | Updated storage recommendations from 2-8°C to 2-25°C. |
| 04 | 09 NOV 2018 | Added use of BD ExK 4-snap |
| 03 | 30 AUG 2018 | Updated open pouch stability. Update to sample pretreatment. Updated reagents section to reflect new packaging. |
| 02 | 13 MAR 2018 | Transition to BioGX EU |
| 01 | 06 APR 2017 | 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 |  | Authorized Representative |

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