BioGX
Molecular Made Easy

Manual

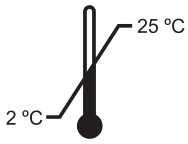
Lesion HSVHD – OSR for BD MAX™

Version 03

REF 400-027-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 Lesion HSVHD – OSR for BD MAX™

INTENDED USE

The BioGX Lesion HSVHD – 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 HSV-1, HSV-2, *Haemophilus ducreyi*, and a DNA sample processing control (abbreviated HSVHD and SPC, respectively). The assay is run on an automated DNA extraction and real-time PCR instrument with Copan ESwab™ or Copan universal transport media (UTM™) from lesion swab collections. Automated extraction of the sample DNA is done using the BD MAX™ ExK™ DNA-3 series extraction kits. The extraction kits contain the SPC DNA so no external addition of SPC is required. The SPC serves as both an extraction control and an internal amplification control (IAC). Each tube of multiplex PCR mix is provided in BioGX proprietary Sample-Ready™ lyophilized format and contains all PCR primers, probes, enzyme, dNTPs, $MgCl_2$, buffers, and other components required for real-time PCR-based analysis of one sample.

SUMMARY AND EXPLANATION

Infection with the herpes simplex virus, commonly known as herpes, can be due to either HSV-1 or HSV-2. HSV-1 is mainly transmitted by oral-to-oral contact and causes infection in or around the mouth whereas HSV-2 is almost exclusively sexually transmitted. *Haemophilus ducreyi* is the causative agent of chancroid and is generally sexually transmitted.

Infection with HSV-1, HSV-2, or *Haemophilus ducreyi* can lead to the formation of blisters or lesions, most commonly in the genitals. For HSV-1, these blisters present most commonly as cold sores or fever blisters on or around the mouth. Lesions caused by HSV-1 and HSV-2 cannot be differentiated by clinical symptoms and require additional laboratory testing to determine the causative agent.

HSV-1 and HSV-2 infections are among the most ubiquitous of human infections. An estimated 90% of all people worldwide will have been infected by one or both of the viruses during their lifetime. On the other hand, *Haemophilus ducreyi* is more rare and generally only observed regularly in areas where access to healthcare is limited. In these developing populations, *Haemophilus ducreyi* is the major cause of skin ulceration in children.

PRINCIPLES OF THE PROCEDURE

The BioGX Lesion HSVHD – 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 HSV-1, HSV-2, *Haemophilus ducreyi* and a Sample Processing Control in four (4) different optical channels of the BD MAX™ System: HSV-2 amplicons are detected in the 530/565 channel, HSV-1 amplicons are detected in the 585/630 channel, *Haemophilus ducreyi* amplicons are detected in the 630/665 channel, and the DNA 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 DNA template. As a result, the fluorophores are separated from their quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the four (4) optical channels used for the BioGX Lesion HSVHD – 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
2	400-027-MAX	**BioGX Lesion HSVHD - OSR for BD MAX™** Sample-Ready™ lyophilized PCR Master Mix containing polymerase, nucleotides, specific molecular primers and probes, Sample Processing Control-specific molecular primers and probe.	12 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™ DNA-3 (BD catalog no. 442822)
  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)
- Copan ESwab™ or Copan universal transport media (UTM™) collection device.
- 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[1] and in Biosafety in Microbiological and biomedical Laboratories.[2]
- 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 2-25 °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™ DNA-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 2-25 °C.

- 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 1 month.

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**.**

NOTE: eUDPs are specific to extraction kit type and are programmed to be used with 3-snap strips unless otherwise indicated. If a 4-snap strip is used it is necessary to modify the eUDP to a 4-snap program by opening the eUDP in "Test Editor" and selecting the corresponding 4-snap extraction strip type in the "Extraction Type" drop down.

Specimen Collection/Transport

Copan ESwab™ and Copan UTM™ specimens should be collected, transported, and stored according to institutional and laboratory standard operating procedures.

Specimen Preparation

Copan ESwab™

Thoroughly vortex the sample prior to addition to the SBT. Add 50 µL of sample/specimen directly to the SBT.

Copan UTM™

Thoroughly vortex the sample prior to addition to the SBT. Add 100 µL of sample/specimen directly to the 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-3 extraction kits with the BioGX Lesion HSVHD – OSR for BD MAX™. DO NOT use BD MAX™ mastermix or the blank 0.3 mL conical tubes from the BD MAX™ ExK™ DNA-3 extraction kit.
3. Load one extraction cartridge into the extraction tray per specimen to be tested.
4. Snap one BD MAX™ ExK™ DNA-3 Extraction Tube into position 1 (Snap-in 1) of each Unitized Reagent Strip. (See Figure 1)

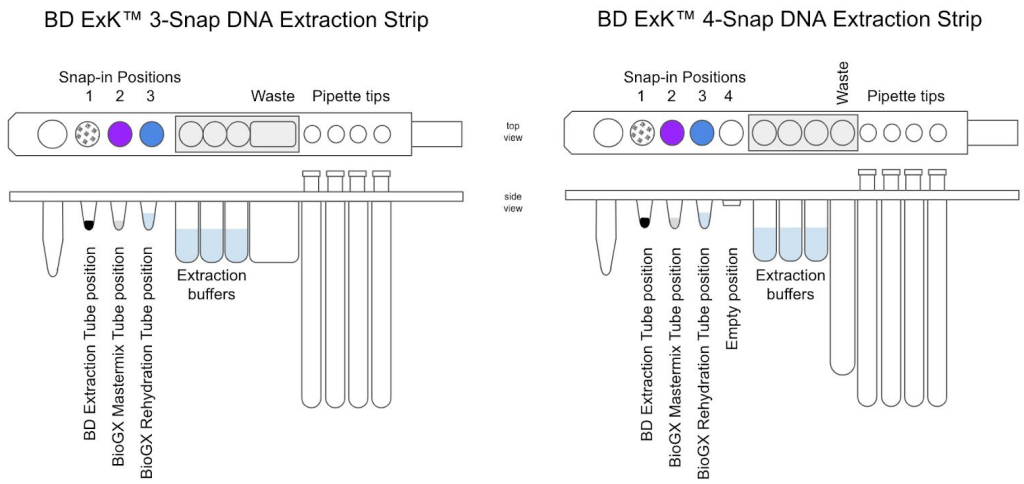

Figure 1 – Diagram of BD MAX™ ExK™ 3-snap and 4-snap Unitized Reagent Strips

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. eUDPs are specific to extraction kit type and are programmed to be used with 3-snap strips unless otherwise indicated.

If a 4-snap strip is used it is necessary to modify the eUDP to a 4-snap program by opening the eUDP in "Test Editor" and selecting the corresponding 4-snap extraction strip type in the "Extraction Type" drop down.

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.

NOTE: If using a 4-snap extraction strip, snap-in position 4 will remain empty.

QUALITY CONTROL

CONTROL

Each BioGX Lesion HSVHD - 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-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
HSV1 POSITIVE	• The HSV-1 target has a Ct within the valid range and endpoint above the minimum setting.
HSV2 POSITIVE	• The HSV-2 target has a Ct within the valid range and endpoint above the minimum setting.
Hducr POSITIVE	• The *Haemophilus ducreyi* target has a Ct within the valid range and endpoint above the minimum setting.
HSV1 NEGATIVE, **HSV2 NEGATIVE,** OR	• The respective target did not amplify and the SPC has a Ct

Hducr NEGATIVE	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*)
INC	● Incomplete Run (with Warning or Error Codes*)

*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 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.
- 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 a 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 Lesion HSVHD – 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 HSV-1, HSV-2, or *Haemophilus ducreyi* resulting in a false negative result with the BioGX Lesion HSVHD – OSR for BD MAX™
- The BioGX Lesion HSVHD – OSR for BD MAX™ requires the use of four (4) optical channels from 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 negative sample matrix (ESwab™ and UTM™) spiked with positive control template. The BioGX Lesion HSVHD - OSR for BD MAX™ was positive for HSV-1, HSV-2, and *Haemophilus ducreyi*.

The BioGX Lesion HSVHD - OSR for BD MAX™ test was run with ATCC MSA-1002 (20 Strain Even Mix Genomic Material) which does not contain genomic DNA for HSV-1, HSV-2, and *Haemophilus ducreyi.* Results were negative for HSV-1, HSV-2, and *Haemophilus ducreyi*.

The BioGX Lesion HSVHD - OSR for BD MAX™ test was run against the Zeptometrix HSV-1 and HSV-2 positive controls (Product Reference: NATHSV1-0004 and NATHSV2-0004, respectively) as well as the Zeptometrix *Haemophilus ducreyi* genomic DNA control (Product Reference: 081736DNA). Controls were diluted to concentrations of 200 copies/SBT or genomic equivalents that represent 200 copies/SBT. All samples were detected as expected.

Analytical and Diagnostic Sensitivity
The analytical sensitivity for the BioGX Lesion HSVHD – OSR for BD MAX™ was determined as follows: Dilution series of positive synthetic DNA samples for each target were added to the SBT in duplicate. Analytical sensitivity (Limit of Detection, LoD) was defined as the lowest concentration at which 95% of all replicates tested positive.

Target	LoD (copies per SBT)
HSV-1	92
HSV-2	82
Haemophilus ducreyi	148

Analytical sensitivity during co-infection was tested by challenging the BioGX Lesion HSVHD – OSR for BD MAX™ in pairs of high concentration (10,000X LOD) of one target against low concentration (1X LOD) of another for all possible pairs in the test. All low concentration targets were positive and were not outcompeted by amplification of the high concentration target.

Reproducibility
The reproducibility study was performed on HSV-1 synthetic target template by three separate technicians independently on two BD MAX™ instruments. All users obtained equivalent results.

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

REFERENCES

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
2. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Choosewood L.C. and Wislon D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.
3. Centers for Disease Control Website, www.cdc.gov, accessed April 13, 2018.
4. Alfa, M. The laboratory diagnosis of *Haemophilus ducreyi*. Can J Infect Dis Med Microbiol 2005;16(1):31-34.
5. Gonzalez-Beiras C et. al. Epidemiology of *Haemophilus ducreyi* Infections. Emerging Inf Dis Jan 2016; 22(1):1-8.
6. Jaishankar D, Shukla D. Genital Herpes: Insights into Sexually Transmitted Infectious Disease. Microb Cell 2016 Jun 27;3(9):438-450.
7. Kularatne, RS et. al. Trends in the relative prevalence of genital ulcer disase pathogens and association with HIV infection in Johannesburg, South Africa, 2007-2015. PLoS ONE 13(4):e0194124.

REVISION HISTORY

Version	Date	Description of Change
03	01 FEB 2019	Updated storage recommendations from 2-8°C to 2-25°C.
02	09 NOV 2018	Added use of BD ExK 4-snap
01	30 AUG 2018	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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