




# Manual

**BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™**

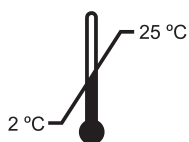
Revision 05

**REF** 400-008-C-MAX

 24 reactions

For *In Vitro* Diagnostic Use

For use with BD MAX™ Open System Reagents on the BD MAX™ System



**BioGX BV**

Science Park 408, 1098 XH Amsterdam, The Netherlands

Phone: +31.20.893.4261

Fax: +31.20.240.9149

**PROPRIETARY NAME**

BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™

**INTENDED USE**

The BioGX Viral Meningitis HSV/VZV – 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 Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Varicella-Zoster Virus (VZV), and a DNA sample processing control (SPC). The assay is run on an automated DNA extraction and real-time PCR instrument with Cerebrospinal Fluid (CSF) samples from individuals at risk for presence of viral meningitis. Automated extraction of the sample DNA is done using the BD MAX™ ExK™ DNA-2 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<sub>2</sub>, 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 to cause infection in or around the mouth whereas HSV-2 is almost exclusively sexually transmitted. Infection with VZV, the common cause of chickenpox, is highly contagious and can be transmitted via droplets, aerosol, or direct contact. Infection with HSV-1, HSV-2, and VZV is widespread throughout the world.

While symptoms of infection with HSV-1, HSV-2, and VZV can be mild, serious complications can occur such as viral meningitis. Viral meningitis is the most common type of meningitis, an inflammation of the tissue that covers the brain and spinal cord. In adults, enteroviruses are the most common cause of viral meningitis followed by HSV-2, VZV, and HSV-1. Both viral and bacterial meningitis are characterized by acute onset of fever, headache, photophobia, and neck stiffness. Viral and bacterial meningitis is difficult to differentiate without lumbar puncture and analysis of cerebrospinal fluid by PCR, and therefore potential cases should be referred to hospital settings.

Herpes simplex viral meningitis is a complication of primary genital herpes (first infection with either virus type in the absence of pre-existing antibodies),

especially with HSV-2. Non-primary genital infection with herpes simplex virus is rarely accompanied by aseptic meningitis. Aseptic meningitis caused by VZV is rarely seen as a complication of primary infection and more commonly seen in association with the reactivation of VZV. Overall, infections of HSV-1, HSV-2, and VZV are common but most people affected have only minor symptoms or no symptoms at all. While viral meningitis can occur at any age, it is most common in young children. In the largest study on the subject, incidence of presumed viral meningitis was 219 per 100,000 in infants under 1 year and 27.8 per 100,000 overall in children under 14.

The BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™ is a real time PCR 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 infection by detecting the presence of HSV-1, HSV-2, and VZV DNA extracted from CSF patient samples collected from individuals at risk of infection.

### **PRINCIPLES OF THE PROCEDURE**

The BioGX Viral Meningitis HSV/VZV – 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, VZV, and the DNA Sample Processing Control in four different optical channels of the BD MAX™ System: VZV amplicons are detected in the 530/565 channel, HSV-2 amplicons are detected in the 585/630 channel, HSV-1 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 DNA, 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 optical channels used for the BioGX Viral Meningitis HSV/VZV – 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	400-008-MAX	<b>BioGX Viral Meningitis HSV/VZV - OSR for BD MAX™</b> 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	<b>Rehydration Buffer Tube (C) Open System Reagents for BD MAX™</b> 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](http://www.biogx.com/eu) or by request.

**EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED**

- BD MAX™ ExK™ DNA-2 (BD catalog no. 442820).  
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).
- CSF 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<sup>6</sup> and in Biosafety in Microbiological and biomedical Laboratories.<sup>7</sup>
- 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 Sample-Ready™ 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-2 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 Manufacturer Recommended Use By Date. Long-term stability studies are ongoing and the Use By 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](http://www.biogx.com/eu) and clicking on the “Product Documentation” and selecting the appropriate platform and product name. eUDPs can also be obtained by emailing BioGX at [eu@biogx.com](mailto: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**

CSF specimens should be collected, transported, and stored according to local, state, federal, international, institutional, and laboratory standard operating procedures.

### **Specimen Preparation**

#### **Cerebrospinal Fluid (CSF) Processing**

Load 200 µL of CSF sample into each SBT to be tested.

**Other Sample Types**

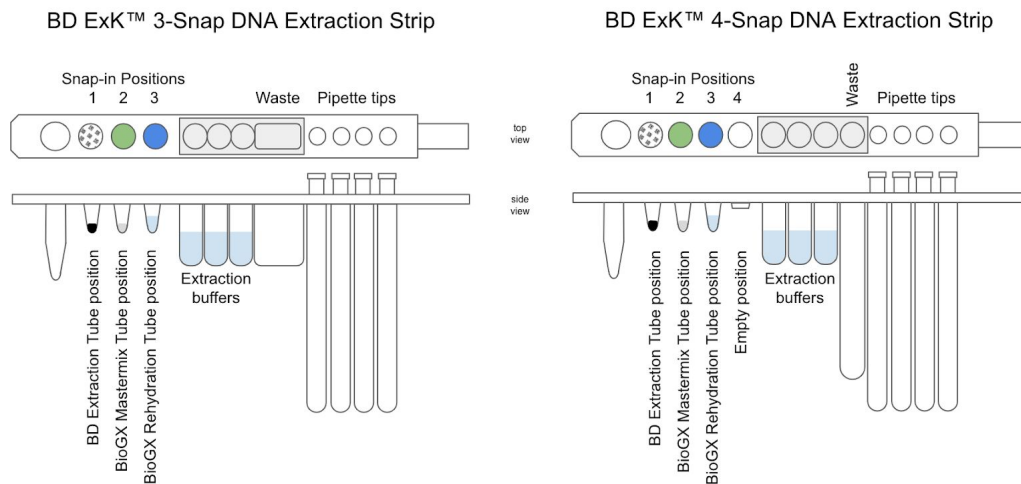


This assay has been optimized for use with the sample types and volumes described above. Use of any other specimen type, collection method, or sample volumes may be inhibitory to the PCR or disrupt extraction without appropriate Guardrail and processing volume adjustments. BioGX does not make claims 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-2 (BD catalog no. 442820) extraction kits with the BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™. **DO NOT** use BD MAX™ mastermix or the blank 0.3 mL conical tubes from the BD MAX™ ExK™ DNA-2 extraction kits.
3. Load one extraction cartridge into the extraction tray per specimen to be tested.
4. Snap one BD MAX™ ExK™ DNA-2 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, >, ^, <) at 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 Viral Meningitis HSV/VZV – 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-2 Extraction Kit. No external addition of SPC template 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.

<b>Results</b>	<b>Interpretation</b>
<b>HSV1 POSITIVE</b>	<ul style="list-style-type: none"> <li>• The HSV-1 target has a Ct within the valid range and endpoint above the minimum setting.</li> </ul>



<b>HSV2 POSITIVE</b>	<ul style="list-style-type: none"> <li>The HSV-2 target has a Ct within the valid range and endpoint above the minimum setting.</li> </ul>
<b>VZV POSITIVE</b>	<ul style="list-style-type: none"> <li>The VZV target has a Ct within the valid range and endpoint above the minimum setting.</li> </ul>
<b>HSV1 NEGATIVE, HSV2 NEGATIVE, OR VZV NEGATIVE</b>	<ul style="list-style-type: none"> <li>The respective target did not amplify and the SPC has a Ct within the valid range and endpoint above the minimum setting.</li> </ul>
<b>UNR</b>	<ul style="list-style-type: none"> <li>Unresolved Result. No target amplification; No SPC amplification.</li> </ul>
<b>IND</b>	<ul style="list-style-type: none"> <li>Indeterminate due to BD MAX™ System failure (with Warning or Error Codes*)</li> </ul>
<b>INC</b>	<ul style="list-style-type: none"> <li>Incomplete Run (with Warning or Error Codes*)</li> </ul>

\*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 the target, the SPC may or may not amplify. This is normal.

### **REPEAT TEST PROCEDURE**

In case of instrument failure, repeat testing can be performed by setting up a new run using 200 µL of the original sample/specimen in a fresh SBT as described above in the Specimen Preparation section.

### **LIMITATIONS OF THE PROCEDURE**

- This product is intended for use with CSF specimens collected using specimen collection 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.
- 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 Viral Meningitis HSV/VZV – 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, and VZV resulting in a false negative result with the BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™.
- The BioGX Viral Meningitis HSV/VZV – 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 Sensitivity**

The analytical sensitivity for the BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™ was determined as follows: Dilution series of positive synthetic DNA samples for each target were added to ExK DNA-2 SBTs 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)	LoD (copies per reaction*)
HSV-1	332	23
HSV-2	262	18
VZV	215	15

\*Assuming 100% efficient extraction on the BD MAX™

The BioGX Viral Meningitis HSV/VZV - OSR for BD MAX™ was tested against the QCMD 2016 Herpes Simplex Virus 1 & 2 EQA Programme and the QCMD 2016 Varicella-Zoster Virus EQA Programme. All core and educational samples reported out were concordant with the expected result.

**QCMD 2016 Herpes Simplex Virus 1 & 2 EQA Programme Results**

Target	Expected Result	Result
HSV-2 CORE Frequently Detected	HSV-2 positive	100% concordant
HSV-1 CORE Frequently Detected	HSV-1 positive	100% concordant
Negative CORE	Negative	100% concordant
HSV-1 CORE Frequently Detected	HSV-1 positive	100% concordant
HSV-2 EDUCATIONAL Detected	HSV-2 positive	100% concordant
HSV-1 CORE Detected	HSV-1 positive	100% concordant
HSV-2 CORE Frequently Detected	HSV-2 positive	100% concordant
HSV-1 CORE Frequently Detected	HSV-1 positive	100% concordant
Negative CORE	Negative	100% concordant
HSV-2 CORE Detected	HSV-2 positive	100% concordant

**QCMD 2016 Varicella-Zoster Virus EQA Programme Results**

Target	Expected Result	Result
VZV Negative CORE	Negative	100% concordant
Varicella-zoster virus (Ellen) EDUCATIONAL Infrequently Detected	VZV positive	100% concordant
Varicella-zoster virus (63/1444) CORE Frequently Detected	VZV positive	100% concordant
Varicella-zoster virus (Ellen) CORE Frequently Detected	VZV positive	100% concordant

Varicella-zoster virus (9/84) CORE Frequently Detected	VZV positive	100% concordant
Varicella-zoster virus (OKA) CORE Frequently Detected	VZV positive	100% concordant
Varicella-zoster virus (9/84) CORE Frequently Detected	VZV positive	100% concordant
Varicella-zoster virus (9/84) CORE Frequently Detected	VZV positive	100% concordant
Varicella-zoster virus (Ellen) CORE Frequently Detected	VZV positive	100% concordant
Varicella-zoster virus (Ellen) CORE Detected	VZV positive	100% concordant

### **Analytical Inclusivity/Exclusivity**

The BioGX Viral Meningitis HSV/VZV – OSR for BD MAX™ primer set is designed to detect the HSV-1, HSV-2, and VZV specific gene targets. Amplicon search *in silico* analysis in BLAST indicates the primers will amplify and the probe will hybridize to all of the ~325 HSV-1 strains, ~142 HSV-2 strains, and ~177 VZV strains. There is a potential cross reactivity of the HSV-2 primer and probe set with 2 sequences of Chimpanzee alpha-1 herpes virus. Other than the sequences mentioned above, none of the other sequences in the BLAST (n) database (as of April 10, 2017) will amplify and report.

### **Reproducibility**

The reproducibility study was performed on VZV synthetic target template by three separate technicians independently on two BD MAX™ instruments. Using one lot of reagents, a series dilution of DNA template was run in ExK DNA-2 between 100,000X LoD and 10<sup>-1</sup> 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<sup>-1</sup> LoD were concordant negative, as expected.

### **Manufacturing Reproducibility**

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
















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Revision	Date	Description of Change
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 reagents section to reflect new packaging. Added new performance data.
02	20 JUN 2018	Updated open pouch stability.
01	07 MAR 2018	Initial Release.

**SYMBOLS**

Symbol	Meaning
	Catalog number
	<i>In vitro</i> 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



**BioGX**BV

Science Park 408, 1098 XH Amsterdam, The Netherlands  
Phone: +31.20.893.4261 Fax: +31.20.240.9149