TECHNICAL
::: NOTE

HSV-1 Detection by the BioFire® FilmArray® Meningitis/ Encephalitis (ME) Panel

Introduction

The purpose of this technical note is to provide a brief overview of the BioFire ME Panel HSV1 assay, and potential causes of discordant results when compared with other methods.

Herpes simplex virus 1 (HSV-1) is a DNA virus belonging to *Herpesviridae* family that establishes latency within sensory neurons in the nervous system. Primary HSV-1 infections usually occur early in childhood and manifest generally as oral lesions[1]. HSV-1 can also cause genital herpes (although more commonly caused by HSV-2), ocular (eye) infections (herpes keratitis), disseminated infections in neonates, and central nervous system (CNS) infections including meningitis and encephalitis (ME).

Detections of HSV-1

Central nervous system infections due to HSV-1 require rapid diagnosis and initiation of antiviral therapy[2]. Several methods with different specificities and sensitivities have been developed for the direct diagnosis of HSV-1 in cerebrospinal fluid (CSF):

- Viral culture allows for viral isolation and strain typing. However, the detection of a suspected herpes virus in culture requires confirmation testing with specific antibodies against HSV-1 and can be time consuming and have a reduced sensitivity compared with PCR-based methods[3].
- Viral antigen detection directly from clinical specimens is a direct relatively low-cost method that does not require the presence of live (viable) virus. It offers a relatively rapid diagnosis; however, the testing does require processing of the sample to concentrate cellular material, staining (generally with a fluorescent monoclonal antibody), expertise in interpretation, and is generally only available in virology laboratories. Unlike multiplex PCR-based method, antigen testing is usually designed to detect one target/assay per test and less sensitive than culture[3]. There are no FDA tests cleared for HSV

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antigen testing directly on CSF, only for culture confirmation. Direct antigen testing of CSF is not recommended due to poor sensitivity.

- PCR-based methods are rapid and allow virus detection and typing in the same test. Due to the poor sensitivity of viral culture for the detection of HSV-1, and HSV-2 in CSF, PCR has replaced viral culture as the gold standard for diagnosis[2,3].

The BioFire® Filmarray® Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-*based in vitro* diagnostic test that has three assays for the detection and differentiation of HSV-1/2. One single assay for the detection of HSV-1 and a combination of two assays for HSV-2. These assays have not been observed to cross-react with each other or with other organisms[4]. Recent studies reported a total of 65 known positive HSV-1 samples that have been tested with the BioFire ME Panel. The BioFire ME Panel detected 62 of the samples[4-9]; two dropouts were due to sample centrifugation[8], which is not consistent with the BioFire ME Panel testing protocol, and one dropout was due to a sample with a low level of nucleic acid (late avg. Ct = 38 on PCR-based LDT)[9]. While the BioFire HSV-1 assay has been developed for optimal sensitivity and specificity for the detection of HSV-1 in patients suspected of a CNS infection; discordant results between the BioFire ME Panel and other methods may occur due to differences in sensitivities and specificities of the assays.

Potential causes of HSV-1 Discrepancy

Apart from laboratory technical errors such as sample handling errors and CSF centrifugation prior to testing, potential sources of discordant HSV-1 results between the BioFire ME Panel and other identification methods include:

- **The presence of HSV-1 strain variation within the sample**

The presence of sequence variants or rearrangements in HSV-1 strain would also contribute to reduction in PCR sensitivity, leading to negative results. Quality Control for Molecular Diagnostics (QCMD) is an independent International External Quality Assessment (EQA)/Proficiency Testing (PT) that provides a wide-ranging quality assessment service primarily focused on molecular infectious diseases. During the QCMD 2017 challenge for CNSI17S and HSVDNA17S, an in-house investigation of samples determined that there is a sequence mismatch between a primer binding site for the BioFire HSV-1 assay and the strain used to prepare these samples. The sequence contained in this strain has not previously been reported in public databases and its frequency is unknown. Moreover, the organism in these samples was present at levels near or below the Limit of Detection (LoD) of the BioFire ME Panel HSV-1 assay.

- **Low levels of HSV-1 within the sample**

Low levels of HSV-1 may cause discrepant results among identification methods. A number of PCR-based methods for the detection and differentiation of HSV-1/2 have been developed with superior sensitivities. Low levels of HSV-1 may be encountered if the CSF is collected early in the course of the disease-resulting in low viral copies, or if the sample is diluted prior to testing[10]. In addition, the majority of HSV-1 replication occurs in the brain, and only a few viral particles are released into the CSF where they can be detected[11]. The level of HSV-1 nucleic acids in patients with encephalitis can be very low at the time of diagnosis (if diagnosed early), as well as for patients with mild clinical symptoms[12].

Recent studies recommend follow-up testing for negative PCR HSV-1 results when the suspicion of the disease is high. The follow-up may include a repeat lumbar puncture followed by testing before declaring the negative results[11,12]. The BioFire® Filmarray® Meningitis/Encephalitis (ME) Panel Instruction Booklet indicates that a negative BioFire FilmArray ME Panel result does not exclude the possibility of CNS infection and should not be used as the sole basis for diagnosis, treatment, or other management decisions. Results from the BioFire ME test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.

- **Detection of clinically false positive HSV within the sample**

Once a person is infected with HSV, the virus establishes latency within sensory neurons in the nervous system. If a person's immune system is compromised due to a concomitant infection, there is a chance that any herpes virus (including HSV-1) may reactivate and potentially be detected in circulating cells present in the CNS, especially when CSF cell counts are elevated[13-15]. However, the virus may not be the cause of the present disease state. A retrospective study by Bhaskaran et al correlated the detection of herpes viruses in CSF with three classifications of disease: definitive, likely and possible true positives[16]. When considering all three categories in total the positive predictive value (PPV) of HSV detection in CSF as the cause of disease was 75% and if considering definitive and likely categories the PPV was 68.75%. They concluded that a positive CSF PCR result must be interpreted with caution as clinical false positives can occur. A positive test result needs to be carefully interpreted in the context of clinical history, epidemiological data, and other data available to the clinician evaluating the patient. Particular caution should be taken when molecular test results appear to be discrepant with the epidemiology and clinical presentations of the patient.

- **Contamination introduced during the testing process**

Contamination can be introduced during collection, handling, storage, sample setup, and testing, which can lead to erroneous results. HSV-1 may be shed from individuals with active or recurrent cold sores[4]. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section. Caution should also be exercised during specimen collection and testing to prevent contamination leading to false positive results.

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Technical Support Contact Information

BioFire is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the BioFire Technical Support team for assistance.

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