FilmArray® ME Panel:
Estimated Limit of Detection for 1st WHO International Standard for Human Cytomegalovirus (HCMV)

1. Introduction

The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis.

Human Cytomegalovirus (HCMV) is one of several viruses associated with meningitis and/or encephalitis that can be detected in CSF by the FilmArray ME Panel.

The World Health Organization (WHO) oversees the development and distribution of International Biological Reference Preparations, which contain a defined biological activity or concentration of an analyte expressed in an internationally agreed unit. In October of 2010, the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques was established [1] and it is currently available through the National Institute for Biological Standards and Control (NIBSC). The HCMV international standard (NIBSC code 09/162) is intended to be used in the standardization of nucleic acid amplification technique (NAT)-based assays for HCMV, allowing for direct comparisons between different methodologies and assays.

This technical note describes estimation of the FilmArray ME Panel Limit of Detection (LoD) for HCMV in international units (IU), as determined by testing of the 1st WHO International Standard for Human Cytomegalovirus. Currently, HCMV is the only analyte detected by the FilmArray ME Panel for which a WHO international standard for nucleic acid-based techniques is available.

2. Estimated LoD for Cytomegalovirus International Standard

FilmArray ME Panel testing was performed to determine an estimated LoD for human Cytomegalovirus (HCMV) in international units (IU). The reference material provided by NIBSC is a lyophilized whole virus preparation of the HCMV Merlin strain [2] (see also Analytical Reactivity
(Table 20 in the FilmArray ME Panel Instruction Booklet [3]). When reconstituted according to the instructions for use provided with the material [4], the concentration of HCMV in the preparation is $5 \times 10^6$ IU/mL. Once reconstituted, contrived samples were prepared by adding a known concentration of the HCMV standard to artificial CSF, followed by serial dilution to obtain five concentrations for testing ($5 \times 10^5$ IU/mL, $5 \times 10^4$ IU/mL, $5 \times 10^3$ IU/mL, $5 \times 10^2$ IU/mL, and $5 \times 10^1$ IU/mL). A total of ten replicates at each concentration were tested using the FilmArray ME Panel on FilmArray and FilmArray 2.0 systems.

Figure 1. FilmArray ME Panel HCMV Detection in Contrived CSF Samples Containing Dilutions of the WHO HCMV International Standard 09/162

As shown in Figure 1, HCMV was detected in 100% of samples when tested at concentrations of $5 \times 10^3$ IU/mL and higher. HCMV was detected in 90% and 50% of samples when tested at concentrations of $5 \times 10^2$ IU/mL and $5 \times 10^1$ IU/mL, respectively. Therefore, the estimated FilmArray ME Panel LoD for HCMV (detection in $\geq 95\%$ of samples) is $5 \times 10^3$ IU/mL.

For comparison, the confirmed LoD for a different HCMV isolate (strain AD-169) is 100 TCID$_{50}$/mL or $4.3 \times 10^5$ copies/mL, as determined by an alternate nucleic acid-based assay (see Table 1 below and the FilmArray ME Panel Instruction Booklet [3]).
Table 1. FilmArray ME Panel LoD for Human Cytomegalovirus

<table>
<thead>
<tr>
<th>ME Panel Test Result</th>
<th>Species/Isolate Tested</th>
<th>LoD Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>CMV, strain AD-169 Zeptometrix 0810003CF</td>
<td>100 TCID₅₀/mL (4.30×10³ copies/mL)</td>
</tr>
<tr>
<td></td>
<td>CMV, strain Merlin NIBSC code 09/162</td>
<td>5×10³ IU/mL*</td>
</tr>
</tbody>
</table>

* Represents the estimated LoD in IU/mL based on the lowest concentration of HCMV international standard detected in at least 95% of the ten replicates tested on FilmArray and FilmArray 2.0 systems.

3. Additional Information

Information about the reference material used in this testing is available from:

WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory

National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG T
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4. References

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 FilmArray Technical Support team for assistance.

BioFire Technical Support
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Phone: +1-801-736-6354, select Option 5 and then Option 1