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

TECHNICAL
::: NOTE

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

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(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 5×10^6 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 (5×10^5 IU/mL, 5×10^4 IU/mL, 5×10^3 IU/mL, 5×10^2 IU/mL, and 5×10^1 IU/mL). A total of ten replicates at each concentration were tested using the FilmArray ME Panel on FilmArray and FilmArray 2.0 systems.

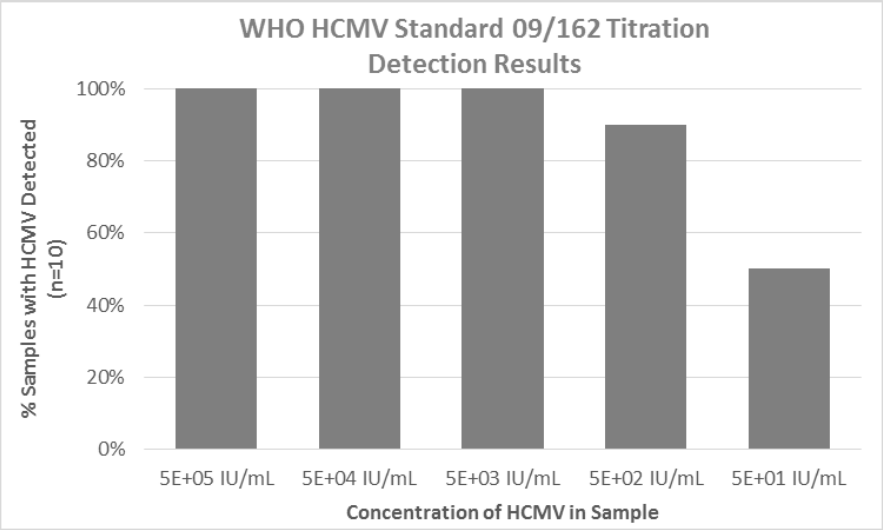

Figure 1. FilmArray ME Panel HCMV Detection in Contrived CSF Samples Containing Dilutions of the WHO HCMV International Standard 09/162

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

For comparison, the confirmed LoD for a different HCMV isolate (strain AD-169) is 100 $TCID_{50}$/mL or 4.3×10^3 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

ME Panel Test Result	Species/Isolate Tested	LoD Concentration
CMV	CMV, strain AD-169 Zeptometrix 0810003CF	100 $TCID_{50}$/mL (4.30×10^3 copies/mL)
	CMV, strain Merlin NIBSC code 09/162	5×10^3 IU/mL[a]

[a] 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
+44 (0)1707 641000

http://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=09/162

4. References

1. Fryer JF. Heath AB, Anderson R, Minor PD and the collaborative study group. Collaborative study to evaluate the proposed 1st WHO International Standard for human cytomegalovirus (HCMV) for nucleic acid amplification (NAT)-based assays. WHO ECBS Report 2010; WHO/BS/10.2138.
2. Dolan A, Cunningham C, Hector RD, Hassan-Walker AF, Lee L, Addison C, Dargan DJ, McGeoch DJ, Gatherer D, Emery VC, Griffiths PD, Sinzger C, McSharry BP, Wilkinson GW, Davison AJ. Genetic content of wild-type human cytomegalovirus. J Gen Virol. 2004;85:1301-12.
3. FilmArray Meningitis/Encephalitis (ME) Panel Instruction Booklet. BioFire Diagnostics, LLC RFIT-PRT-0276-01 October 2015.
4. 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques. NIBSC Code: 09/162. Instructions for Use. Version 6.0 Dated 9/10/2014.

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
Email: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5 and then Option 1

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