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French External Quality Assessment (EQA) Scheme for Molecular Detection of Human Herpesviruses (HHVs) in Cerebrospinal Fluid (CSF) Samples

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on behalf of the French HSV Study Group

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INTRODUCTION

Herpes simplex viruses 1 and 2 (HSV-1/2), varicella-zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and Epstein-Barr virus (EBV) are highly prevalent HHVs that may be responsible for central nervous system (CNS) infections with potential life-threatening course. Therefore, accurate detection of HHVs by sensitive molecular testing in CSF from patients with possible CNS infection is highly recommended. The regular assessment of the performances of diagnostic tests in laboratories is required. In this context, a French national EQA program was organized for the molecular detection of HHVs in CSF samples.

METHODS

The 2018 HHV EQA panel comprised 4 constructed samples prepared by dilution of viral stocks in a HHV-negative CSF matrix: HSV-1 (strain KOS), HSV-2 (strain gHSV-2), VZV (strain Oka), CMV (strain AD169), EBV (clinical strain), and/or HHV-6B (strain MAR) (**Table 1**). The 50 participating laboratories were asked to return results and a questionnaire on technical and methodological information detailing the procedure employed within 6 weeks.

Table 1. 2018 HHV EQA panel composition

Sample ID	Viruses	Detection*
1	HSV-1 (laboratory strain KOS)	FD
	HHV-6B (laboratory strain MAR)	IF
2	VZV (laboratory strain Oka)	FD
	EBV (clinical strain)	FD
3	gHSV-2 (laboratory strain gHSV-2)	FD
	EBV (clinical strain)	D
4	CMV (laboratory strain AD169)	FD
	HHV-6B (laboratory strain MAR)	FD

**Each sample is assigned a detection frequency based on the overall qualitative results: frequently detected (FD), detected (D), and infrequently detected (IF) correspond to >95%, between 65% and 95%, and <65% of datasets with correct result, respectively.*

RESULTS

A total of 58 datasets were received from the 50 participants; 4 laboratories returned multiple datasets. Most laboratories used automated platforms for nucleic acid extraction (86%; 37% used easyMAG® platform) (**Table 2**).

Table 2. Workflows employed for cerebrospinal fluid (CSF) analysis

Input CSF volume (range)	25 µL to 400 µL
Sample-to-result methods	FilmArray® Torch - BioMérieux MDX Liaison® - DiaSorin
Manual extraction methods	QIAamp® - Qiagen NucleoSpin® - Macherey - Nagel
Automated extraction methods	easyMag® / Emag® - BioMérieux EZ1® - Qiagen m2000® SP - Abbott MagNA Pure 96® - Roche Diagnostics MagNA Pure Compact® - Roche Diagnostics QIAsymphony® SP - Qiagen DuplicaPREP® - Euroclone
Amplification platforms	ABI® 7300, 7500, 7900 - Applied Biosystems RotorGene® 6000 - Qiagen RotorGene® Q - Qiagen CF96 Touch®- BioRad LightCycler® (480 and 2.0) - Roche Diagnostics m2000 RealTime PCR - Abbott Mx3005®P qPCR system - Stratagene SmartCycler® - Cepheid

Most laboratories used commercial assays for molecular detection: 91% for HSV-1/2 and VZV PCR (40% used HSV-1/HSV-2/VZV® R-gene kit [BioMérieux]), 92% for CMV PCR (45% used CMV® R-gene kit [BioMérieux]), 92% for EBV PCR (54% used EBV® R-gene kit [BioMérieux]), and 80 % for HHV-6 PCR (80% used HHV-6® R-gene kit [BioMérieux]). For HHV-6, only 31% of the laboratories used variant A/B-specific real-time PCR. Several platforms were indexed for PCR amplification (Table 2). Interestingly, 1 laboratory used a panpathogen assay based on metagenomic next-generation sequencing (NextSeq®500 - Illumina). Qualitative PCR was used for HSV-1/2 (76%) and VZV (78%), and quantitative PCR for CMV (63%), EBV (75%), and HHV-6 (67%). For CMV and EBV, 22% and 23% of the participating laboratories expressed the quantitative results using international units, respectively. Globally, high HHV detection performances were obtained, except for HHV-6B (sample 1) and EBV (sample 3), probably due to low viral loads (< 300 copies/mL) (**Table 1** and **Figure 1**).

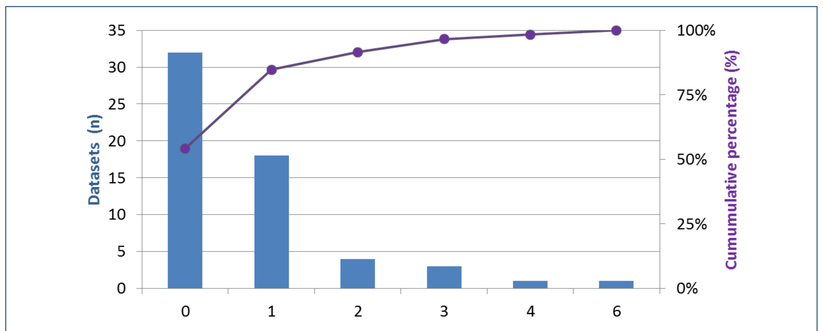

Figure 1. Sum quantitative of scores for all participants
The scoring system used for qualitative detection was based on the sample status: no points for correct detection; 1 point when participant returned a negative result for an 'infrequently detected' panel sample; 2 points when participant returned a negative result for an 'detected' panel sample; 3 points when participant returned an incorrect genotype result or a negative result for an 'frequently detected' panel sample.

CONCLUSIONS

Marked heterogeneity was observed for nucleic acid extraction and amplification procedures. However, results revealed encouraging analytical performances of HHV molecular assays in CSF samples.

French HSV Study Group

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