External quality assessment for herpes simplex virus drug resistance testing: A French feasibility study

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INTRODUCTION

Among immunocompromised patients, herpes simplex virus (HSV) infections are associated to morbidity and mortality, and the therapeutic management may be challenging with the emergence of HSV resistance to currently used antivirals acyclovir (ACV) and foscanet (FOS). The prevalence of HSV infections caused by ACV-resistant isolates in immunocompromised patients ranges from 3.5% to 11% [1]. The diagnosis of HSV drug resistance can be performed using either phenotypic assays, by the measurement of the antiviral 50% effective concentration (EC_{50}), and/or genotypic assays, by the sequencing of UL23 thymidine kinase (TK) and UL30 DNA polymerase genes. However, the standardization of these methods is required in the era of quality assurance. In an attempt to compare the results for HSV drug resistance between different virology laboratories, an external quality assessment (EQA) pilot study was organized in France.

MATERIALS AND METHODS

In 2014 and 2015, 3 French virology laboratories (Lab 1, 2, and 3) participated to this EQA pilot study. Each year, one laboratory coordinator prepared and sent to the 2 other participating laboratories a blinded EQA panel of 3 samples consisting of either HSV clinical isolates, previously characterized for drug susceptibility to ACV and FOS, or HSV-negative samples. The EQA panel was thereafter analyzed phenotypically (2014 and 2015) and genotypically (2015 only) by the 3 laboratories according to their local routine procedures. Participants were instructed to describe the methodologies of their assays, and to report the following results: EC_{50} for ACV and FOS, resistance mutations identified within TK and DNA polymerase, and the corresponding interpretations (i.e., susceptibility or resistance to antivirals). Results were asked to be reported to the laboratory coordinator within 4 weeks of the receipt of the panels.

Table 1. Phenotypic results of HSV drug-resistance EQA testing 2014

	Lab 1		Lab 2		Lab 3		
	ACV EC_{50} (µM)	FOS EC_{50} (µM)	ACV EC_{50} (µM)	FOS EC_{50} (µM)	ACV EC_{50} (µM)	FOS EC_{50} (µM)	Interpretation
HSVDR14-01	<1	<66	1.3	87	6.5	151	ACV-S FOS-S
HSVDR14-02	-	-	-	-	-	-	HSV negative
HSVDR14-03	>50	<66	14.5	120	183	125	ACV-R FOS-S

ACV, acyclovir; EC_{50}, 50% effective concentration; FOS, foscarnet; R, resistant; S: susceptible.

Table 2. Phenotypic results of HSV drug-resistance EQA testing 2015

	Lab 1		Lab 2		Lab 3		
	ACV EC_{50} (µM)	FOS EC_{50} (µM)	ACV EC_{50} (µM)	FOS EC_{50} (µM)	ACV EC_{50} (µM)	FOS EC_{50} (µM)	Interpretation
HSVDR15-01	<1	<66	0.3	180	<0.8	77.6	ACV-S FOS-S
HSVDR15-02	>50	96	32	95	30	81	ACV-R FOS-S
HSVDR15-03	18	<66	8	31	20	<62.5	ACV-R FOS-S

ACV, acyclovir; EC_{50}, 50% effective concentration; FOS, foscarnet; R, resistant; S: susceptible.

Table 3. Genotypic results of HSV drug-resistance EQA testing 2015

	Lab 1			Lab 3		
	UL23 TK*	UL30 DNA polymerase*	Interpretation	UL23 TK*	UL30 DNA polymerase*	Interpretation
HSVDR15-01	None	None	ACV-S FOS-S	None	None	ACV-S FOS-S
HSVDR15-02	R281Stop	None	ACV-R FOS-S	R281Stop	None	ACV-R FOS-S
HSVDR15-03	R221C	None	ACV-R FOS-S	R221C	ND	ACV-R

* Only resistance mutations are indicated.
ACV, acyclovir; FOS, foscarnet; ND, not done; R, resistant; S, susceptible; TK, thymidine kinase.

RESULTS

Results from all participants were generated using laboratory-developed techniques (LDT) for both phenotypic (Labs 1, 2, 3) and genotypic (Labs 1 and 3) antiviral resistance assays, as previously described [2-4]. The deadline for submitting the results was met by all 3 laboratories.

The EQA panel 2014 consisted of one drug-sensitive HSV-2 isolate (HSVDR14-01), one HSV-negative sample (HSVDR14-02), and one ACV-resistant HSV-2 isolate (HSVDR14-03). Only phenotypic analysis was performed by the 3 participating laboratories. As presented in Table 1, results from all laboratories were 100% concordant and matched the expected results.

The EQA panel 2015 included one drug-sensitive HSV-1 isolate (HSVDR15-01), one ACV-resistant HSV-1 isolate (HSVDR15-02), and one ACV-resistant HSV-2 isolate (HSVDR15-03). Once again, all phenotypic results, corresponding to the expected ones, were 100% concordant (Table 2). The genotypic analysis performed by Labs 1 and 3 provided similar results: HSV resistance to ACV was the consequence of a nucleotide mutation in UL23 gene leading to the creation of a premature stop codon at position 281 of TK for sample HSVDR15-02, and of the amino acid change R221C for sample HSVDR15-03. (Table 3).

CONCLUSION

This French EQA pilot study for assessing the proficiency of laboratories in the detection of HSV drug resistance demonstrates the feasibility of the organization of a multicenter quality control for interlaboratory comparison. Despite the use of different LDT, the participating laboratories provided concordant results and similar interpretations for both phenotypic and genotypic analyses. However, the standardization of some parameters may be required, such as the antiviral EC_{50} values for phenotypic assays, or the choice of the HSV reference sequence for genotypic analysis. The next French EQA study aims at being extended to all virology laboratories performing HSV drug resistance analysis. Moreover, an international HSV drug resistance typing EQA pilot study will be organized by the Quality Control for Molecular Diagnostics (QCMD, Glasgow, Scotland) in 2016.

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