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Validation of Simplexa™ HSV 1 & 2 Direct and Simplexa™ VZV Direct kits for HSV and VZV detection in low-volume cerebrospinal fluid samples

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INTRODUCTION

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV) are major causes of viral meningoencephalitis [1-2]. Rapid and accurate detection of viral genomes in cerebrospinal fluid (CSF) samples is therefore mandatory for the management of patients. However, low-volume CSF may be available for molecular testing in virology laboratories.

AIM

We evaluated the sample-to-result Simplexa™ HSV 1 & 2 Direct assay and Simplexa™ VZV Direct assay on the LIAISON® MDX platform with the use of 25 µL of sample volume, instead of 50 µL as recommended by the manufacturer DiaSorin.

METHOD

Cycle threshold (Ct) values obtained from 25 µL and 50 µL of sample volume tested in parallel were compared for 60 HSV-positive and 60 VZV-positive different samples: 35 positive CSFs from patients, 37 negative CSFs spiked with different quantities of ATCC HSV-1, HSV-2, or VZV strains, and 48 QCMD samples. Reproducibility of Simplexa™ HSV 1 & 2 Direct assay and Simplexa™ VZV Direct assay was evaluated by intra-assay and inter-assay comparisons with the use of 25µL of sample volume.

RESULTS

1- Samples tested

Table 1. Samples used for the comparison of Ct values obtained from 25 µL and 50 µL of volume tested

	HSV-positive samples (n=60)	VZV-positive samples (n=60)
CSF from patients	17	18
CSF spiked with ATCC viral strains[a]	18	19
QCMD samples[b]	25	23

[a]ATCC –VR-1493: HSV-1, strain KOS; ATCC-VR-734: HSV-2, strain G; ATCC-VR-1367: VZV, strain Ellen.

[b]QCMD samples from Herpes simplex virus DNA EQA programmes and Varicella-Zoster virus DNA EQA, years 2018 and 2019.

2- Difference of Ct values

All 120 positive samples were detected using either 25 µL or 50 µL, leading to a concordance of 100%. No PCR inhibition was observed. The difference of Ct values obtained with the 2 volumes tested (ΔCt [25-50]) was below or equal to 1.0 for 101 (84%) samples and ranged from 1.1 to 1.6 for the 19 (16%) remaining samples (Figure 1). Mean ΔCt [25-50] (SD) values were -0.1 (0.6), -0.1 (0.8), and -0.2 (0.7) for HSV-1, HSV-2, and VZV, respectively.

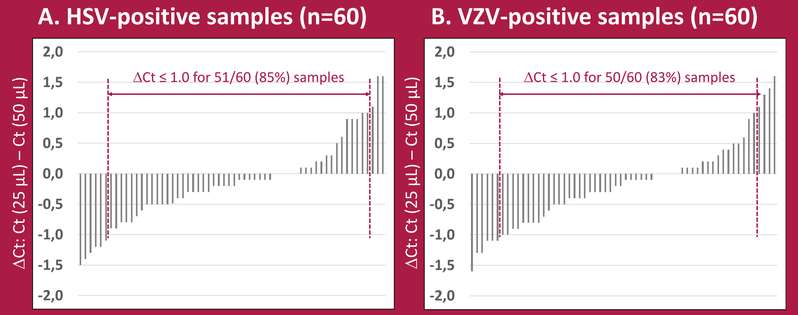

Figure 1. Distribution of difference of Ct values (ΔCt [25-50]) for samples tested in parallel for HSV (A) and VZV (B) using either 25 µL or 50 µL of volume.

3- Assay reproducibility

Intra-assay variability

Method: Simplexa™ Positive Controls for HSV-1, HSV-2, and VZV were tested 8 times with a volume of 25 µL within the same Direct Amplification Disk (DAD) during the same run on the LIAISON ® MDX.

Results: Coefficients of variation (CVs) for intra-assay variability were 3.0%, 3.0%, and 1.9% for HSV-1, HSV-2, and VZV, respectively.

Inter-assay variability

Method: Simplexa™ Positive Controls for HSV-1, HSV-2, and VZV were tested 10 consecutive times once a week with the volume of 25 µL on the LIAISON ® MDX.

Results: Coefficients of variation (CVs) for inter-assay variability were 1.7%, 1.0%, and 1.3% for HSV-1, HSV-2, and VZV, respectively.

CONCLUSIONS

The results obtained in this study allow the validation of the performances of Simplexa™ HSV 1 & 2 Direct kit and Simplexa™ VZV Direct kit for the detection of HSV-1, HSV-2, and VZV genomes in CSFs with the use of 25µL of sample with no loss of sensitivity. Our results are in line with those previously obtained by Espy *et al.* [3]. This protocol will be useful for clinical laboratories to perform molecular tests with low-volume CSF samples, especially for pediatric patients.

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