

# Evaluation of the DiaSorin Molecular Simplexa VZV Direct Assay for Direct Detection of Varicella Zoster Virus in Cerebrospinal Fluid in Adult and Pediatric Populations



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## Background

Detection of Varicella zoster virus (VZV)-nucleic acid in cerebrospinal fluid (CSF) can determine the etiology and help to guide therapy in patients with encephalitis. Currently, there are no FDA-cleared single analyte assays for detection of VZV DNA in the CSF. In this study, we evaluated the performance of two assays using DiaSorin equipment and analyte specific reagents, **Simplexa™ VZV Direct** and **VZV LDT DAD assay**, for detection of VZV DNA in clinical samples submitted for VZV testing in the CSF without the need for a separate nucleic acid extraction step.

Simplexa™ VZV Direct assay is now FDA-cleared for use with CSF samples.

## Methods

We retrospectively tested 59 CSFs that were either submitted and approved for VZV testing in the Microbiology Laboratory at the Hospital of University of Pennsylvania between August-December 2018 (N=33) or banked samples from the Infectious Disease Diagnostic Laboratory at Children's Hospital of Philadelphia (n=17) and ARUP Laboratories (N=10). For the Simplexa™ VZV Direct Assay, we added 50 µL of Simplexa reaction mix and 50 µL of sample to the direct amplification disc. For VZV LDT DAD assay, we made the master mix by spiking 1 µL of VZV primer/probe into the Simplexa HSV 1 & 2 Direct assay reaction mix. To determine specificity, we spiked into clinical samples that were strongly positive for cytomegalovirus (n=3), human herpesvirus 6 (n=3), Epstein-Barr virus (n=3), Enterovirus (n=3), herpes simplex virus types 1 (n=2) and herpes simplex virus types 2 (n=2) into CSF negative for VZV.



Figure 1. DiaSorin's Liaison MDX and Amplification Disc

## Results

Expected Value (IU/mL)	Average Ct	# Replicates	% Positives
<b>Simplexa™ VZV Direct</b>			
1000	35.29	10	100
500	36.6	10	100
250	38.0	10.0	90.0
125	40.6	9.0	77.8
<b>VZV LDT DAD assay</b>			
1000	35.3	10	100
500	36.6	10	90
250	38.0	10	80
125	40.6	10	70

Table 1. Limit of Detection of the Simplexa™ VZV Direct Assay (2,524 c/mL) and VZV LDT DAD Assay (985 c/mL).

## Results

- The limit of detection for the Simplexa™ VZV Direct Assay and VZV LDT DAD assay were 2,524 and 985 copies/mL, respectively.
- The positive agreement for the Simplexa™ VZV Direct Assay was 100% (12/12) and for VZV LDT DAD assay was 91.7% (11/12). One sample that was undetectable by the VZV LDT DAD assay was weakly positive (Ct of 39 by the Simplexa™ VZV Direct Assay).
- The negative agreement for both VZV Direct and VZV LDT DAD assay was 100% (47/47).
- The specificity for both the assays was 100% (n=16) as there was no cross-reactivity with other Herpesviruses and Enterovirus in the CSF.
- Reproducibility was demonstrated by serial testing of identical positive (n= 14) and negative samples (n=15) during the period of the study. The mean Ct and coefficient of variation for the Simplexa™ VZV Direct Assay was 31.4 and 3.2; VZV LDT DAD assay was 28.9 and 3.9, respectively.

## Results

Virus	Source	Ct or VL (c/mL) of Source	Dilution	Simplexa™ VZV Direct Assay	VZV LDT DAD Assay
CMV-1	Blood	26.88	1:10	Negative	Negative
CMV-2	Urine	23.76	1:10	Negative	Negative
CMV-3	Blood	26.04	1:10	Negative	Negative
HHV6-1	TONS	21.76	1:10	Negative	Negative
HHV6-2	Serum	24.98	1:10	Negative	Negative
HHV6-3	Blood	23.26	1:10	Negative	Negative
EBV-1	Blood	21.98	1:10	Negative	Negative
EBV-2	Blood	27.85	1:10	Negative	Negative
EBV-3	Blood	24.49	1:10	Negative	Negative
EV-1	SLSW	20.45	1:10	Negative	Negative
EV-2	SLSW	27.6	1:10	Negative	Negative
EV-3	Vesicle	21.87	1:10	Negative	Negative
HSV-1 (Zepto)	N/A	50,000	1:5	Negative	Negative
HSV-2 (Zepto)	N/A	50,000	1:5	Negative	Negative
HSV-2	CSF	35	Neat	Negative	Negative
HSV-2	CSF	34	Neat	Negative	Negative

Table 2. Specificity of the Simplexa™ VZV Direct Assay and VZV LDT DAD Assay against other Herpesviruses and Enterovirus. Positive patient samples or control material (HSV-1, 2; Zeptomatrix) were spiked into a CSF matrix and tested for VZV with Simplexa™ VZV Direct Assay and VZV LDT DAD Assay.

## Conclusions

- Our data shows that the DiaSorin Molecular Simplexa™ VZV Direct assay for direct detection of VZV DNA in CSF has excellent performance characteristics for clinical testing.
- During this study period, the majority of patients only required HSV and VZV testing and this assay represents a good alternative for targeted detection of VZV in the CSF of both adult and pediatric population.

**We would like to thank DiaSorin Molecular for providing reagents for this study. DiaSorin had no role in the design, execution, or interpretation of the study.**