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Comparison of the Simplexa HSV 1 & 2 Direct Kit and Laboratory-Developed Real-time PCR Assays for Herpes Simplex Virus Detection

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BACKGROUND

Rapid detection and differentiation of herpes simplex viruses (HSV) is important for patient management and treatment, especially in cases of HSV meningoencephalitis.

The FDA-cleared Simplexa HSV 1 & 2 Direct Assay (Focus Diagnostics, Cypress, CA) is a rapid, sample-to-result, microfluidic real-time PCR system for the direct detection and differentiation of HSV types 1 and 2 from cerebral spinal fluid specimens (CSF) that have not undergone nucleic acid extraction.

Objective

To compare the performance of the Simplexa HSV 1 & 2 Direct assay to that of the University of Washington laboratory-developed real-time PCR tests (LDTs) for the detection of HSV1 and HSV2 DNA in CSF.

METHODS

CSF (n=230)

- Patients seen at University of Washington Medical Center (UW)
  - **81** residual CSF, stored at -80ºC.
    - Collected March, 2009-August, 2013
    - **26** previously positive for HSV
  - **80** CSF tested prospectively
    - Collected February-April, 2014
- Neonatal herpes study (NN)
  - **69** residual CSF, stored at -80ºC.
    - Collected 1981-1989
    - 34 patients
    - Median age 24 days (range, 2-258 days)
- All samples tested by Simplexa Direct and LDTs on same day.

LDTs

- Automated nucleic acid extraction
  - Roche MagNA Pure 96 or MagNA Pure LC
  - Input vol 200 uL, elution vol 100 uL
- Real-time PCR amplification
  - Primers and TaqMan probe target HSV glycoprotein B gene
  - 7500 Real Time PCR System (Applied Biosystems)
  - 95% LOD = 250 viral copies/mL

Simplexa Direct

- 50 uL reagent mix, 50 uL CSF
- Direct Amplification Disc
- 3M Integrated Cycle
- Primers target HSV polymerase gene
- Results ~1 hour

RESULTS

HSV 1 Detection by LDT and Simplexa Direct

Table 1. 161 UW patients

	Simplexa Direct			
LDT	Pos	Neg	Invalid	Total
Pos	3	0	0	3
Neg	**1**	154	3@	158
Total	4	154	3	161

Concordance (%)	**97.5**
Kappa	**0.59**
Positive agreement (%)	**100**

Table 2. 66* NN patients

	Simplexa Direct			
LDT	Pos	Neg	Invalid	Total
Pos	11#	**2**	0	13
Neg	**2**	51	0	53
Total	13	53	0	66

Concordance (%)	**94.0**
Kappa	**0.81**
Positive agreement (%)	**85.0**

Table 3. Discrepant **HSV1** results (PCR Ct value)

LDT	Simplexa Direct
neg	39.2
neg	33.4
neg	35.4
38.7	neg
41.2	neg

HSV 2 Detection by LDT and Simplexa Direct

Table 4. 161 UW patients

	Simplexa DIrect			
LDT	Pos	Neg	Invalid	Total
Pos	21	**1**	0	22
Neg	0	136	3@	139
Total	21	137	3	161

Concordance (%)	**97.5**
Kappa	**0.90**
Positive agreement (%)	**95.5**

@3 (1.3%) of 230 clinical CSF were invalid by Simplexa Direct.

Table 5. 66* NN patients

	Simplexa Direct			
LDT	Pos	Neg	Invalid	Total
Pos	21#	**3**	0	24
Neg	**3**	39	0	42
Total	24	42	0	66

Concordance (%)	**91.0**
Kappa	**0.80**
Positive agreement (%)	**88.0**

*3 samples pos, but not typed, by LDT. Of these, 2 were HSV2 pos and 1 was neg by Simplexa Direct.
#8 specimens from 5 patients were positive for both HSV1 and HSV2 by LDT and Simplexa Direct.

Table 6. Discrepant **HSV2** results (PCR Ct value)

LDT	Simplexa Direct
neg	36.4
neg	39.0
neg	40.1
33.7	neg
38.3	neg
32.7	neg
36.7	neg

Simplexa Direct Performance

Table 7. Sensitivity and specificity of Simplexa Direct for detection of HSV1 and HSV2 compared to LDTs

	Performance (%)	
Target	Sensitivity	Specificity
HSV1	87.5	97.2
HSV2	91.3	96.7

Table 8. Relative sensitivity of Simplexa Direct and LDTs assessed using serial dilutions of HSV1 and HSV2 cultures tested in 8 replicates

		LDT		Simplexa Direct	
Target	Dil	mean Ct	% pos	mean Ct	% pos
HSV1	10^{-6}	33.6	100	36.0	100
	10^{-7}	37.9	100	39.3	50
HSV2	10^{-6}	32.7	100	36.6	100
	10^{-7}	35.5	100	40.0	75

Table 9. Reproducibility of Simplexa Direct assessed by testing a panel of 5 samples 8 times

Target	N	Mean Ct	%CV
HSV1	8	22.9	1.8
HSV1	8	31.9	3.9
HSV2	8	23.6	2.4
HSV2	8	33.0	2.8
Internal control	40	30.2	0.7

SUMMARY and CONCLUSIONS

- The Simplexa Direct performed well compared with our established LDTs for the detection of HSV1 and HSV2 in CSF.
- The majority of targets missed by both assays had high Ct values (>35), indicating low viral loads in the specimens.
- When tested using serially diluted samples, the relative sensitivity of Simplexa Direct was similar to that of LDT for HSV2. For HSV1, LDT showed slightly better sensitivity than Simplexa Direct.
- Compared to our LDTs, which require several hours for nucleic acid extraction and PCR amplification, Simplexa Direct, which provides results in ~ 1 hour, will allow for more rapid antiviral treatment and patient management.

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Focus Diagnostics donated the Simplexa Direct HSV 1 & 2 kits for this study.