CSF Sample-to Answer Validation Studies Using Focus Diagnostics' Simplexa™ HSV 1 & 2 Direct Assay

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Abstract

Objectives: Simplexa™ HSV 1 & 2 Direct assay is a sample-to-answer multi-analyte detection system performed on the 3M Integrated Cycler. Cerebrospinal fluid (CSF) specimens are loaded directly onto a Direct Amplification Disc without any separate specimen preparation or extraction steps. Results are generated in approximately 60 minutes. Simplexa HSV 1 & 2 Direct detects and differentiates herpes simplex viruses (HSV) 1 and 2. The objective of this study was to evaluate and compare the performance of the Simplexa HSV 1 & 2 Direct assay with clinical and sequencing results that utilize conventional nucleic acid extraction procedures.

Methods: Limit of detection (LoD) studies were performed to determine the analytical sensitivity of the assay. A panel of bacteria and viruses was tested to evaluate cross reactivity. A panel of potentially interfering substances was tested to determine whether any inhibition was observed. A reproducibility study panel was tested at 3 separate sites. Clinical performance was determined by testing blinded CSF clinical specimen panels with Simplexa HSV 1 & 2 Direct and comparing the results to the consensus of physician's assessment based on clinical data which included neural imaging results when available, and results from two different sequencing assays.

Results: LoD studies showed that Simplexa HSV 1 & 2 Direct detected HSV-1 strains at less than 50 $TCID_{50}$/mL and HSV-2 strains at less than 25 $TCID_{50}$/mL. None of the organisms tested for cross reactivity showed positive detection. No inhibition or interference was observed from any of the substances tested. All reproducibility samples results were ≥ 90% from 3 different testing sites. Positive and negative clinical agreements were 100% (15/15) and 98.1% (209/213) for HSV-1, and 100% (39/39) and 93.1% (176/189) for HSV-2.

Conclusion: The Simplexa HSV 1 & 2 Direct assay was able to detect and differentiate HSV-1 and HSV-2 from clinical CSF specimens without any apparent loss in sensitivity when compared to physician's assessment and sequencing methods that utilized conventional nucleic acid extraction. The Simplexa HSV 1 & 2 Direct assay and the 3M Integrated Cycler instrument provides a compact system for rapid sample to result detection of HSV-1 and HSV-2 directly from human CSF samples in approximately 1 hour. The Simplexa HSV 1 & 2 Direct assay is FDA cleared and CE marked.

Methods

HSV Strains: The following strains were used: HSV-1 McIntyre (Virapur, San Diego, CA), HSV-1 HF (Virapur), HSV-2 G, (Virapur), and HSV-2 MS (Virapur).

Limit of Detection (LoD): The LoD for each HSV viral stock was determined as the lowest concentration with ≥95% detection in human CSF (Golden West Biologicals, Temecula, CA).

Methods (Continued)

Extraction, Real-time PCR Amplification and Detection: Simplexa HSV 1 & 2 Direct Kit, MOL2150 (Focus Diagnostics, Cypress, CA), contains all reagents for on-board extraction and real-time PCR assay. 50 µL of Simplexa HSV 1 & 2 reaction mix was loaded into the reaction port and 50 µL of sample was loaded into the sample port on the Direct Amplification Disc (DAD). All testing was performed using the 3M Integrated Cycler instrument (3M, St. Paul, MN). Data collection and analysis were performed with Integrated Cycler Studio software.

Reproducibility: Three separate test sites each tested 30 replicates of the following contrived panel in human CSF: HSV-1 Low Positive (HSV-1 McIntyre at 1X LOD), HSV-1 Medium Positive (HSV-1 McIntyre at 4X LOD), HSV-2 Low Positive (HSV-2 G at 1X LOD, HSV-2 Medium Positive (HSV-2 G at 4X LOD).

Sensitivity and Specificity: A panel of 228 de-identified CSF clinical specimens (Focus Diagnostics) were evaluated for HSV-1 and HSV-2 using the Simplexa HSV 1 & 2 Direct assay. Simplexa results were compared to the consensus of two sequencing assays and neural imaging result (2 out of 3 for consensus). The PCR region utilized by both sequencing assays was different than the target region detected by the Simplexa HSV 1 & 2 Direct kit.

Cross Reactivity: The cross reactivity panel consisted of industry equivalent 10^6 CFU/mL of bacteria or 10^5 $TCID_{50}$/mL of virus in human CSF.

Inhibition/interference: The interference panel was contrived with HSV-1 or HSV-2 at 4-fold above LoD concentration. Each substance was spiked into the HSV-1 or HSV-2 human CSF contrived samples and tested using Simplexa HSV 1 & 2 Direct.

Results

Limit of Detection: HSV-1 (McIntyre and HF strains) LoD was ≤40 $TCID_{50}$/mL. HSV-2 (G and MS strains) LoD was ≤20 $TCID_{50}$/mL in human CSF.

Table 1. HSV Limit of Detection

Strain	Concentration ($TCID_{50}$/mL)	Detection Rate (# Detected/ # Total)	# of replicates with Ct detected	Mean ± SD
HSV-1 McIntyre	2.5	*93.8% (30/32)*	*30*	*37.9 ± 1.03*
	5	**96.9% (31/32)**	*31*	*37.1 ± 1.17*
HSV-1 HF	20	*87.5% (28/32)*	*29*	*37.8 ± 0.98*
	40	**96.9% (31/32)**	*31*	*36.9 ± 0.90*
HSV-2 G	0.625	*65.6% (21/32)*	*21*	*39.3 ± 0.88*
	1.25	**96.9% (31/32)**	*31*	*38.3 ± 0.74*
HSV-2 MS	10	*90.6% (29/32)*	*29*	*37.6 ± 0.92*
	20	**100% (32/32)**	*32*	*37.2 ± 1.03*

Limit of Detection determined as lowest concentration with ≥95% detection

Results (Continued)

HSV Reproducibility: HSV-1 and HSV-2 medium and low contrived panels were detected ≥90% at 3 different test sites. Standard deviations were ≤1.14. Percent Coefficients of Variation were ≤3.1.

Table 2. HSV Reproducibility

Panel	Total %Agreement with Expected Results	Quantitative Summary of Reproducibility										
		Mean	Inter-Site		Inter-Day		Inter-Run/Operator		Intra-Run		Total	
		Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HSV-1 Low Positive	100.0% (90/90)	36.3	0.76	2.1	0	0	0.41	1.1	0.74	2	1.14	3.1
HSV-1 Med Positive	100.0% (90/90)	34	0.72	2.1	0.12	0.3	0.34	1	0.41	1.2	0.91	2.7
HSV-2 Low Positive	90.0% (81/90)	38.3	0	0	0	0	0.45	1.2	1	2.6	1.1	2.9
HSV-2 Med Positive	100.0% (90/90)	34.8	0.23	0.7	0	0	0.27	0.8	0.46	1.3	0.58	1.7

SD = Standard Deviation
%CV = Percent Coefficients of Variation

Positive and Negative Agreements: Positive and negative agreements between the consensus of neural imaging and 2 sequencing assay results for HSV-1 and HSV-2 were >93%.

Table 3. HSV Positive and Negative Percent Agreement

Agreement	HSV-1	HSV-2
Sensitivity/ PPA	*100.0%(15/15)*	*100.0%(39/39)*
	95% CI: 79.6 to 100.0%	*95% CI: 91.0 to 100.0%*
Specificity/ NPA	*98.1%(209/213)*	*93.1%(176/189)*
	95% CI: 95.3 to 99.3%	*95% CI: 88.6 to 95.9%*

PPA = Positive Percent Agreement
NPA = Negative Percent Agreement

Table 4. HSV Clinical Agreement

Simplexa™ Results	Consensus: Neural Imaging and 2 Sequencing Assay Results (2 out of 3)			
	HSV1	HSV2	Not Detected	Total
HSV-1	*15*	*0*	*4* [a]	**19**
HSV-2	*0*	*39*	*13* [b]	**52**
Not Detected	*0*	*0*	*157*	**157**
Total	**15**	**39**	**174**	**228**

[a] 4 HSV-1 discrepant specimens: One sample was HSV-1 positive by neural imaging (Simplexa HSV-1 Ct =34.7). One sample was HSV-1 positive by one of the two sequencing assays (Simplexa HSV-1 Ct =30.3). Two samples were HSV-1 negative by neural imaging and both sequencing assays (Simplexa HSV-1 Ct =36.2 and 37.1).

[b] 13 HSV-2 discrepant specimens: Two samples were HSV-2 positive by neural imaging (Simplexa HSV-2 Ct =34.9 and 35.6). Nine samples were HSV-2 positive by one of the two sequencing assays (Simplexa HSV-2 Ct were between 34.7-38.5). Two samples were HSV-2 negative by neural imaging and both sequencing assays (Simplexa HSV-2 Ct =35.2 and 36.1).

Results (Continued)

Cross Reactivity: No cross-reactivity was detected with the pathogens tested in Table 5.

Table 5. HSV Cross Reactivity Pathogens Tested in Human CSF

Adenovirus 1	HIV-1	Parainfluenza Virus 2
Adenovirus 7	HIV-2	Parainfluenza Virus 3
BKV	HHV-6	Parvovirus
Citrobacter freundii	HHV-7	Poliovirus
Citrobacter koseri	HHV-8	*Proteus mirabilis*
Cryptococcus neoformans	Influenza A	*Pseudomonas aeruginosa*
CMV	Influenza B	Rabies
Dengue	JCV	Rhinovirus
Encephalomyocarditis virus	*Klebsiella pneumoniae*	Rotavirus
Enterobacter aerogenes	*LA Crosse encephalitis*	St. Louis Encephalitis Virus
Enterovirus 71	*Listeria monocytogenes*	*Staphylococcus aureus*
EBV	Measles	*Streptococcus agalactiae*
Escherichia coli O157H7	Mumps	*Streptococcus pneumoniae*
Haemophilus influenzae type B	*Mycobacterium tuberculosis*	*Toxoplasma gondii*
Haemophilus influenzae non-B	*Naegleria fowleri*	VZV
Hepatitis B	*Neisseria meningitidis*	WNV
Hepatitis C	Parainfluenza Virus 1	

Substance Interference: No interference was detected with the substances tested in Table 6.

Table 6. Potential Interferents Tested in Human CSF

Potential Interferent	Concentration Tested	HSV Concentration Tested
Whole Blood	10% (v/v)	HSV-1 (McIntyre) 20 $TCID_{50}$/mL (4x LoD) or HSV-2 (G) 5 $TCID_{50}$/mL (4x LoD)
Albumin (protein)	10 mg/mL	
Casein (protein)	10 mg/mL	
Acyclovir (Acycloguanosine)	2.5 mg/mL	
Betadine (topical antiseptic)	5% (v/v)	
White Blood Cells	$5.5x10^8$ WBC/mL	
Hemoglobin	0.625 mg/mL	

Conclusions

- Simplexa HSV 1 & 2 Direct is a simple and rapid molecular test. Simplexa HSV 1 & 2 Direct provides answers in ~60 minutes, without requiring a separate extraction step.
- Simplexa HSV 1 & 2 Direct was capable of directly detecting and differentiating HSV-1 and HSV-2 from un-extracted CSF specimens, with performance comparable to sequencing and neural imaging.
- Simplexa HSV 1 & 2 Direct is FDA cleared and CE marked and is currently available for sale.

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