Simplexa™ HSV 1 & 2 Direct

REF MOL2150
Rev. E

A real-time PCR assay intended for the *in vitro* qualitative detection and differentiation of HSV-1 and/or HSV-2 viral DNA.

For *in vitro* Diagnostic Use
CLIA – Moderate Complexity

INTENDED USE

The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct is intended for use on the 3M Integrated Cycler instrument for the qualitative detection and differentiation of HSV-1 and HSV-2 DNA in cerebrospinal fluid (CSF) samples from patients suspected of herpes simplex virus (HSV) infections of the central nervous system (CNS). This test is intended as an aid in the diagnosis of HSV-1 and HSV-2 infections of the CNS.

Negative results do not preclude HSV-1 or HSV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

The assay is not intended for use as a donor screening test. The assay is for professional use only.

SUMMARY AND EXPLANATION

HSV-1 and HSV-2 are members of the alpha-herpesviridae subfamily. HSV is an enveloped virus with a capsid containing viral DNA. Although HSV-1 and HSV-2 are closely related, the two viruses are serologically and genetically distinct.[1,2]

Encephalitis is inflammation of the brain associated with clinical evidence of neurologic dysfunction. Of the pathogens reported to cause encephalitis, the majority are viruses.[3] In general, the most commonly identified etiologies in the United States are herpes simplex virus, West Nile virus, and the enteroviruses, followed by other herpesviruses.[3]

HSV causes about 5–10% of all encephalitis cases, and is one of the most common causes of identified sporadic encephalitis globally.[3] HSV encephalitis occurs in all ages, and during all seasons. HSV-1 encephalitis is more common in adults; and HSV-2 encephalitis is more common in neonates.[3] One study reported a neonatal herpes rate of 1 case per 3,200 live births in the U.S.[4]

Clinical features involved with HSV encephalitis include fever, hemicranial headache, language and behavioral abnormalities, memory impairment, and seizures.[3]

The utility of nucleic acid amplification testing (e.g., PCR) of CSF specimens has greatly increased the ability to diagnose infections of the CNS, especially viral infections caused by the herpesviruses.[3] CSF cultures are generally of limited value in the determination of the viral causes of encephalitis but are very important in the diagnosis of bacterial and fungal infections. [3] The Infectious Diseases Society of America (IDSA) recommends herpes simplex PCR testing on all CSF specimens in patients with encephalitis. For encephalitis patients with a negative herpes simplex PCR result, consideration should be given to repeating the test 3–7 days later for patients demonstrating a compatible clinical syndrome or temporal lobe localization on neuroimaging.[3]

The American Congress of Obstetricians and Gynecologists (ACOG) has recognized that HSV PCR is 1.5-4 times more sensitive than viral culture, that samples for PCR are easier to maintain, and that PCR is the test of choice in the diagnosis of infections of the central nervous system (encephalitis and meningitis).[5]

PRINCIPLES OF THE PROCEDURE

The Simplexa™ HSV 1 & 2 Direct assay system is a real-time PCR that enables the direct amplification, detection and differentiation of HSV-1 and/or HSV-2 DNA from unprocessed CSF specimens without nucleic acid extraction. The system consists of the Simplexa™ HSV 1 & 2 Direct assay, the 3M Integrated Cycler (with 3M Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ HSV 1 & 2 Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify HSV-1, HSV-2 and internal control targets. Well conserved regions of the HSV-1 and HSV-2 DNA polymerase genes are targeted to identify HSV-1 and HSV-2 DNA respectively in the specimen. An internal control is used to detect PCR failure and/or inhibition.

MATERIALS PROVIDED

The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct assay contains sufficient reagents for 24 reactions. Upon receipt, store at -10 to -30 ºC (do not use a frost-free freezer). Each vial contains sufficient material for a single reaction. Use within 30 minutes of thawing.

Kit Description

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ HSV 1 & 2 Direct Reaction Mix	MOL2151	REAG	A	RM	Brown	24	1/24	50 µL

Component Description

Kit Component	Contents
Simplexa™ HSV 1 & 2 Direct Reaction Mix (RM)	DNA polymerase, buffer, dNTPs, template DNA (Internal Control) dye-labeled fluorescent probe-primers specific for detection of HSV-1 and/or HSV-2 and for the DNA Internal Control.

Target	Probe Fluorophore (Dye)	Excitation (nm)	Emission (nm)	Targeted Gene
HSV-1	CFR610	590	610	HSV-1 DNA polymerase
HSV-2	FAM	495	520	HSV-2 DNA polymerase
DNA Internal Control	Q670	644	670	NA

Simplexa™ HSV 1 & 2 Kit Barcode Card	Assay specific parameters.

MATERIALS SUPPLIED SEPARATELY

1. Direct Amplification Disc Kit (REF MOL1455)
    a) Direct Amplification Discs for use on the 3M Integrated Cycler

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 3M Integrated Cycler with 3M Integrated Cycler Studio Software version 6.0 or higher
2. Simplexa™ HSV 1 & 2 Positive Control Pack (REF MOL2160)
3. 50 µL fixed volume pipette (VWR Signature™ Fixed Volume Ergonomic High-Performance Pipette Model VWR FE50 or equivalent)
4. Sterile, nuclease-free disposable pipette tips with filters (Extra Long tips ≥ 91 mm are recommended for pipetting directly from primary collection tubes).
5. Freezer (manual defrost) at -10 to -30 °C (for kit component and specimen frozen storage)
6. Refrigerator at 2 to 8 °C (for specimens)
7. Disposable, powder-free gloves

RECOMMENDED MATERIALS

1. Synthetic CSF Golden West Biologicals Catalog number OH1030-S, SeraCare Catalog number HSP-515 or equivalent for use as a No Template Control (NTC).

REAGENT HANDLING AND STORAGE

1. Store reagents at -10 to -30 °C (do not use a frost-free freezer).
2. Allow reagents to thaw at room temperature (approximate range 18 to 25 °C) before use.
3. Do not use kits or reagents beyond their expiration dates.
4. After removing Reaction Mix from freezer storage, initiate the test within 30 minutes.
5. Do not vortex the Reaction Mix.
6. Do not refreeze the Reaction Mix.

7.

WARNINGS AND PRECAUTIONS

1. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
2. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
3. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
4. Treat all specimens and discs as capable of transmitting infectious agents. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.[6,7]
5. Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results.
6. Assay setup should be performed at room temperature (approximate range 18 to 25 °C).
7. Use fixed volume pipettes or equivalent for sample and Reaction Mix.
8. Avoid touching the under side of the foil that will be in contact with the wells and disc surface.
9. To prevent potentially erroneous results, make sure that the sample is added to the Sample input well.
10. Finish loading and applying adhesive foil cover to one set of Sample and Reaction wells before opening the foil of adjacent set(s)s of Sample and Reaction wells.
11. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer.
12. Do not attempt to remove adhesive foil cover from wedges that have been used or attempt to re-use Sample and Reaction ports that have been used in previous runs.
13. Discs may be reused until all 8 wedges have been used. Dispose of used discs without detaching foil cover in biohazardous waste container.
14. After each use store DAD discs flat with the numbered foil side up.
15. Reaction Mix contains > 1% glycerol, which may cause irritation upon inhalation or skin contact. Upon inhalation or skin contact, first aid measures should be taken.
16. If kit packaging or contents appear to be broken or damaged do not use and contact Focus Diagnostics. Contact information is on the last page of this document.
17. **The spectral matrix must be installed in each 3M Integrated Cycler and should not be changed unless an updated QR code for the instrument is provided by Focus Diagnostics. The spectral matrix is unique to each 3M Integrated Cycler. The spectral matrix was provided with the 3M Integrated Cycler instrument on the cover of the 3M Integrated Cycler Hardware Manual. If the matrix label will not scan or cannot be found contact Focus Diagnostics. The contact information is on the last page of this document.**
18. **Not installing or changing the spectral matrix can result in false results.**

INSTRUCTIONS FOR USE

A. SPECIMEN COLLECTION

Acceptable specimen type is CSF. Specimens should be transported on ice and stored at 2 to 8°C for up to 7 days post collection. If there is a greater than 7 days delay before processing of the specimen, store specimen at -70° C.

B. REAL-TIME PCR INSTRUMENT SETUP

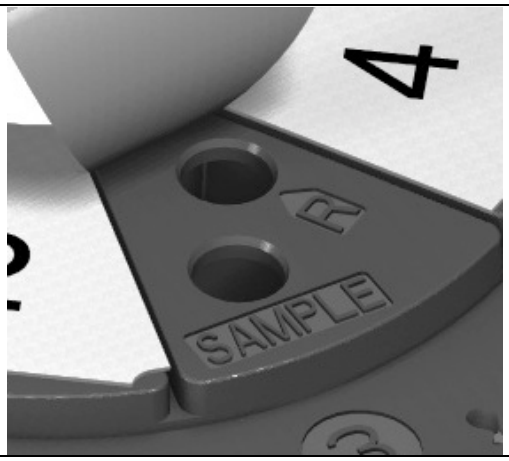
1. Refer to the 3M Integrated Cycler Operator Manual for details on how to configure the 3M Integrated Cycler Studio Software to add an assay definition set up and analyze runs on the 3M Integrated Cycler.

C. DIRECT AMPLIFICATION DISC LOADING AND REAL-TIME PCR AMPLIFICATION

NOTE: No sample extraction is needed prior to PCR amplification step.

1. Select samples that need to be tested.
2. Thaw Reaction Mix vials at room temperature (approximate range 18 to 25 °C). Thaw one Reaction Mix vial for each sample or control to be tested.
3. Scan the barcode on the Simplexa™ HSV 1 & 2 Direct Reaction Mix vial or barcode card.
4. Scan the disc barcode on the Direct Amplification Disc (DAD).
5. Scan or type in each sample identifier.
6. For one wedge at a time, peel the adhesive foil back to expose the Sample (SAMPLE) and Reaction (R) wells without completely removing the adhesive foil cover (Figure 1 & 2). Avoid touching the under side of the foil that will be in contact with the wells and disc surface.
7. Ensure that the Reaction Mix is completely thawed. Briefly spin down the tubes as needed. (Do not vortex the Reaction Mix).
8. Use the fixed volume pipette to transfer 50 µL of the Reaction Mix into Reaction (R) well.
9. Use the fixed volume pipette to transfer 50 µL of sample or control; pipette sample or control into Sample well (SAMPLE).
10. Cover the wedge sealing the wells with the peeled adhesive foil, pressing down firmly near the edge of the wedge. If the original foil is torn do not load the wells in the wedge. Instead load another wedge.
11. Tear off the tab portion of the foil cover along the perforation.
12. Repeat steps 6 to 11 for the next sample(s).

13. Load the sealed DAD into the 3M Integrated Cycler and start the run.

Figure 1 - Disc with pre-use foil lifted from Sample and Reaction Wells for wedge #3	Figure 2 –Sample [Sample] and Reaction [R] Wells
	

NOTES *(for informational purposes - no user action/interpretation required)*:

1. Focus Diagnostics kits may contain version numbers for Assay Definitions. If the version number exists, it will be appended to the Assay Definition i.e. 'Sample IVD Assay.2'. When multiple versions exist, the software automatically uses the assay definition associated with the scanned lot number.

QUALITY CONTROL

Simplexa™ HSV 1 & 2 Positive Control Pack (MOL2160) may be used as an external control for Quality Control (QC) testing, training or proficiency testing. Synthetic CSF is recommended as a No Template Control (NTC). Quality control ranges have been established as indicated in the table below. If the controls are not within these parameters, patient results should be considered invalid and the assay repeated. Focus recommends testing controls once per day. Each laboratory should establish its own QC ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice. Refer to the Simplexa™ HSV 1 & 2 Positive Control package insert (PI.MOL2160) for instructions on testing the positive control.

Expected Control Results

Control Type	HSV-1	HSV-2	DNA Internal Control (DNA IC)
Simplexa™ HSV 1 & 2 Positive Control[1]	Detected	Detected	Not applicable[2]
No Template Control (NTC)	Not Detected	Not Detected	Valid

[1] Typical Ct values for the Positive Control range between 25 to ≤40.

[2] Detection of the Simplexa™ DNA Internal Control (DNA IC) is not required for a valid result when HSV is detected.

RESULTS

Upon completion of the run, the software automatically calculates and displays results.

1. For each accession ID (Sample ID) entered, the software displays a result ("Detected", "Not Detected", "Invalid" or "EC500") for HSV-1 and HSV-2.
    a. **"Detected"** result points to the presence of HSV-1 and/or HSV-2 DNA in the patient sample.
    b. **"Not Detected"** result points to the absence of HSV-1 and/or HSV-2 DNA in the patient sample.
    c. **"Invalid"** result points to the inability to determine presence or absence of HSV-1 and/or HSV-2 DNA in the patient sample. This result may be due to 1) DNA Internal Control (DNA IC) failure, or 2) failure to detect sufficient specimen. The sample needs to be re-tested. See "Invalid Results" section below.
    d. **"EC500"** result points to a data quality error for the particular viral analyte(s). The software was unable to determine a valid amplification for that analyte(s). The sample should be re-tested.
2. Print the report as needed.
    a. Export the results as needed

INVALID RESULTS

In case of an "Invalid" result, re-test the sample with a new Reaction Mix vial from the same kit or a new kit. If the problem is unresolved, contact Focus Diagnostics Technical Services department.

LIMITATIONS

1. For *in vitro* diagnostic use.
2. In the United States, this product is intended for use in healthcare facilities with a minimum CLIA certification of moderate complexity.
3. Results from this test must be considered in conjunction with the clinical history, epidemiological data and other laboratory information available to the clinician evaluating the patient.
4. The detection of viral nucleic acid is dependent upon proper sample collection, transport, handling and storage. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
5. The prevalence of viral infections may affect the test's predictive value.
6. Negative results do not rule out HSV infections of the CNS and should not be used as the sole basis for treatment or other patient management decisions.
7. False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
8. For encephalitis patients with a negative herpes simplex PCR result, consideration should be given to repeating the test 3–7 days later for patients demonstrating a compatible clinical syndrome or temporal lobe localization on neuroimaging.[3]
9. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
10. A positive result by this test cannot rule out infections caused by other viral or bacterial pathogens. Viral nucleic acids may persist *in vivo* independent of virus viability. Detection of target analyte(s) does not imply that the corresponding viruses are infectious or are the causative agent for clinical symptoms.
11. When very high levels of HSV-2 are present with very low levels of HSV-1, the signal from the HSV-1 reaction may not be adequate to be detected, due to competitive interference.
12. The prevalence of viral infections may affect the test's predictive value.
13. This test is a qualitative test and does not provide the quantitative value of detected virus present.
14. The performance of this test has not been established for screening of blood or blood products for the presence of HSV or for use with samples other than CSF.
15. The performance of this test has not been established for immunocompromised individuals.
16. The performance of this test has not been established for monitoring treatment of HSV infection of the CNS.
17. Information on the Simplexa™ HSV 1 & 2 Direct Reaction Mix vial can only be transferred into the 3M Integrated Cycler Studio through a bar-code scanner. If the scanner is not working, or if you are unable to transfer the information for any reason, contact Focus Diagnostics Technical Services.

PERFORMANCE CHARACTERISTICS

EXPECTED VALUES

The observed expected values of HSV-1 and HSV-2 in the prospective population of the Simplexa™ HSV 1 & 2 Direct assay's clinical study varied between institutions and are shown in the table below.

Sample Population	Sample Collection Site	No. of Samples	Expected Values Based on Simplexa™ HSV1/2 Direct Assay		
			HSV-1	HSV-2	Not Detected
Prospective	1	31	0.0% (0/31)	6.5% (2/31)	93.5% (29/31)
	7	24	4.2% (1/24)	0.0% (0/24)	95.8% (23/24)
	8	94	4.3% (4/94)	5.3% (5/94)	90.4% (85/94)
	9	15	0.0% (0/15)	0.0% (0/15)	100.0% (15/15)
	All	**164**	**3.0% (5/164)**	**4.3% (7/164)**	**92.7% (152/164)**

Age Category	Prospective - Number of Samples by Simplexa™ HSV1/2 Direct Assay											
	Combined				Female				Male			
	HSV -1	HSV -2	Not Detected	All	HSV-1	HSV-2	Not Detected	All	HSV-1	HSV-2	Not Detected	All
From Birth to 1 month of age		1	9	10			6	6		1	3	4
>1 month to 2 years of age		1	25	26			8	8		1	17	18
>2 years to 12 years of age			9	9			6	6			3	3
>12 years to 21 years of age		1	11	12		1	4	5			7	7
>21 years to 60 years of age	4	4	65	73	3	1	35	39	1	3	30	34
>60 years of age	1		33	34	1		20	21			13	13
All	**5**	**7**	**152**	**164**	**4**	**2**	**79**	**85**	**1**	**5**	**73**	**79**

CLINICAL AGREEMENT

A total of 219 CSF samples were prospectively or retrospectively collected from patients with signs and symptoms of Herpes Simplex Virus (HSV) central nervous system (CNS) infection by eight external sites. All sites sent the frozen samples to Focus Diagnostics. Two aliquots were prepared from each sample and the samples were then blinded for testing. One aliquot was sent to an external testing site while the other aliquot was retained at Focus Diagnostics for sequencing. Five external sites performed testing with the Simplexa™ HSV 1 & 2 Direct assay which included four of the collection sites. Focus Diagnostics performed testing on the samples using PCR followed by analysis with two bi-directional sequencing assays. Results from the Simplexa™ HSV 1 & 2 Direct assay were compared to the results from two bi-directional sequencing assays (comparator). Neural imaging/clinical impression results documented in a case report form (CRF) as determined by the patient's attending physician and other clinical information such as chemistries, bacterial culture, MRI/CT scans and in-house PCR results were collected for all patients. The Final Diagnosis takes into consideration all of the laboratory findings and the results of a PCR test performed locally along with clinical presentation of the patient. No dual positive (both HSV-1 Positive and HSV-2 Positive) samples were found.

The table below shows the distribution of the different clinical parameters collected from patients who were confirmed positive and negative by the comparator.

Parameter	Patients which are positive by the comparator for HSV (Number and %)	Patients which are negative by the comparator for HSV (Number and %)
MRI results suggestive of HSV infection	18/65 (27.7%)	9/154 (5.8%)
MRI results not suggestive of HSV infection	43/65 (66.2%)	121/154 (78.6%)
MRI results not available	4/65 (6.2%)	24/154 (15.6%)
Final Diagnosis positive for HSV infection	61/65 (93.8%)	33/154 (21.4%)
Final Diagnosis negative for HSV infection	4/65 (6.2%)	121/154 (78.6%)
Bacteria culture positive	2/65 (3.1%)	4/154 (2.6%)
Bacteria culture negative	63/65 (96.9%)	145/154 (94.2%)
Bacteria culture not performed	0/65 (0.0%)	5/154 (3.2%)

The following tables summarize the results of the Simplexa™ HSV 1 & 2 Direct assay's agreement with the comparator (results of two sequencing PCR assays). Three hundred (300) base-pair dideoxy DNA sequencing assays were validated for two different HSV gene targets. SYBR Green PCR using extracted patient sample DNA was used to test all samples. Amplicons from positive samples were used for bidirectional sequencing. Sequence requirements had a phred quality score ≥20 (for each base) and a continuous read length ≥200 and a 2x coverage ≥180. Sequence alignments were analyzed against the GenBank database using the BLAST search program to determine if the patient sample was positive for HSV-1 or HSV-2.

HSV-1: Prospective Samples			
Simplexa™ Results	Comparator: Two PCR/SEQ		
	Detected	Not Detected	Total
Detected	3	2*	5
Not Detected	0	159	159
Total	3	161	164
PPA	100.0%(3/3) 95% CI: 43.8 to 100.0%		
NPA	98.8%(159/161) 95% CI: 95.6 to 99.7%		

*1/2 sample had a final diagnosis of meningitis from the chart information.

HSV-2: Prospective Samples			
Simplexa™ Results	Comparator: Two PCR/SEQ		
	Detected	Not Detected	Total
Detected	6	1[##]	7
Not Detected	1[#]	156	157
Total	7	157	164
PPA	85.7%(6/7) 95% CI: 48.7 to 97.4%		
NPA	99.4%(156/157) 95% CI: 96.5 to 99.9%		

[#]Sample was collected from patient with a final diagnosis of meningitis from the chart information.

[##]Sample was collected from patient with a final diagnosis of meningitis from the chart information.

Note: PPA: Positive Percent Agreement; NPA: Negative Percent Agreement

Fifty five (55) retrospective/preselected HSV positive (as determined by the collection sites) samples from four sites were collected between 2004 and 2013 and confirmed positive by the comparator were used to supplement the prospective study to evaluate the sensitivity of the Simplexa™ HSV 1 & 2 Direct. The results are shown in the table below.

Retrospective/ Preselected Positive Samples – Positive Percent Agreement (PPA)		
Simplexa™ Results	**Comparator: Two PCR/SEQ**	
	HSV-1	HSV-2
HSV-1	13	0
HSV-2	0	42
Not Detected	0	0
Total	**13**	**42**
	HSV-1	**HSV-2**
PPA	100.0% (13/13) 95% CI: 77.2 to 100.0%	100.0%(42/42) 95% CI: 91.6 to 100.0%

REPRODUCIBILITY

Reproducibility for the Simplexa™ HSV 1 & 2 Direct assay was evaluated. Three investigative sites assessed the device's inter-site, inter-day and inter/intra-assay reproducibility. Each of the laboratories tested positive control and a panel of five contrived sample pools including a low (approximately 1 times LoD) and medium positive (approximately 4 times LoD) for each analyte and a high negative. The high negative sample contained a small amount of HSV-1 and HSV-2, and it was designed to be negative approximately 95% of the time. The assays were performed in triplicate on five different days. Each site had two operators who each assayed the entire sample panel and positive control once per day, for a total of two sets of data per day. Combined results for all sites are presented in the tables below.

	Sample	**Site – 1**			**Site – 2**			**Site – 3**			**Total % Agreement with Expected Results**	**95% CI**
		% Agreement with Expected Results	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**		
HSV-1 Result	HSV-1 Low Positive	100.0% (30/30)	36.9	2.0	100.0% (30/30)	35.4	2.2	100.0% (30/30)	36.6	2.7	100.0% (90/90)	95.9% to 100.0%
	HSV-1 Medium Positive	100.0% (30/30)	34.6	1.6	100.0% (30/30)	33.2	1.2	100.0% (30/30)	34.3	1.8	100.0% (90/90)	95.9% to 100.0%
	HSV-2 Low Positive	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (90/90) [a]	95.9% to 100.0%
	HSV-2 Medium Positive	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (90/90) [a]	95.9% to 100.0%
	High Negative	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (90/90) [a]	95.9% to 100.0%
	Positive Control	100.0% (30/30)	30.7	1.5	100.0% (30/30)	30.6	1.2	100.0% (30/30)	30.2	1.4	100.0% (90/90)	95.9% to 100.0%
	Total Agreement	**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (540/540)**	**99.3% to 100.0%**

a) Expected Results of HSV-2 Low Positive, HSV-2 Medium Positive and High Negative samples are “Negative” for HSV-1.

	Sample	Site – 1			Site – 2			Site – 3			Total % Agreement with Expected Results	95% CI
		% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV		
HSV-2 Result	HSV-1 Low Positive	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (90/90) [b]	95.9% to 100.0%
	HSV-1 Medium Positive	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (90/90) [b]	95.9% to 100.0%
	HSV-2 Low Positive	96.7% (29/30)	38.1	2.9	90.0% (27/30)	38.4	3.2	83.3% (25/30)	38.3	2.4	90.0% (81/90)	82.1% to 94.6%
	HSV-2 Medium Positive	100.0% (30/30)	35.0	1.3	100.0% (30/30)	34.6	1.8	100.0% (30/30)	35.0	1.4	100.0% (90/90)	95.9% to 100.0%
	High Negative	93.3% (28/30) [b]	39.7	0.2	96.7% (29/30) [b]	38.4	NA	96.7% (29/30) [b]	39.1	NA	95.6% (86/90) [b]	89.1% to 98.3%
	Positive Control	100.0% (30/30)	30.1	0.9	100.0% (30/30)	30.6	1.1	100.0% (30/30)	30.0	1.2	100.0% (90/90)	95.9% to 100.0%
	Total Agreement	**98.3% (177/180)**			**97.8% (176/180)**			**96.7% (174/180)**			**97.6% (527/540)**	**95.9% to 98.6%**

b) Expected Results of HSV-1 Low Positive, HSV-1 Medium Positive and High Negative samples are "Negative" for HSV-2.

	Sample	Site – 1			Site – 2			Site – 3			Total % Agreement with Expected Results	95% CI
		% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV		
DNA IC Result	HSV-1 Low Positive	100.0% (30/30)	29.6	0.7	100.0% (30/30)	30.0	1.7	100.0% (30/30)	29.1	0.6	100.0% (90/90)	95.9% to 100.0%
	HSV-1 Medium Positive	100.0% (30/30)	29.6	0.8	100.0% (30/30)	30.1	1.2	100.0% (30/30)	29.1	0.6	100.0% (90/90)	95.9% to 100.0%
	HSV-2 Low Positive	100.0% (30/30)	29.4	0.7	100.0% (30/30)	30.1	1.0	100.0% (30/30)	29.1	0.7	100.0% (90/90)	95.9% to 100.0%
	HSV-2 Medium Positive	100.0% (30/30)	29.4	0.9	100.0% (30/30)	30.0	1.3	100.0% (30/30)	29.2	1.6	100.0% (90/90)	95.9% to 100.0%
	High Negative	100.0% (30/30)	29.4	0.8	100.0% (30/30)	30.1	1.3	100.0% (30/30)	29.2	1.3	100.0% (90/90)	95.9% to 100.0%
	Positive Control	100.0% (30/30)	29.6	1.0	100.0% (30/30)	30.0	1.1	100.0% (30/30)	29.2	0.8	100.0% (90/90)	95.9% to 100.0%
	Total Agreement	**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (540/540)**	**99.3% to 100.0%**

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) was determined for the Simplexa™ HSV 1 & 2 Direct assay using quantified stocks of HSV-1 and HSV-2 serially diluted into negative human CSF matrix. LoD was determined to be the lowest concentration that could be detected positive $\geq$ 95% of the time.

Virus Strain	LoD Concentration ($TCID_{50}$/mL)	Qualitative Results (#Detected/#Total)	Mean Ct ± SD (from Detected Replicates only)
HSV-1 McIntyre	5	31/32	37.1 ± 1.17
HSV-1 HF	40	31/32	36.9 ± 0.90
HSV-2 G	1.25	31/32	38.3 ± 0.74
HSV-2 MS	20	32/32	37.2 ± 1.03

ANALYTICAL REACTIVITY/CROSS REACTIVITY

Analytical Reactivity

The analytical reactivity of the Simplexa™ HSV 1 & 2 Direct assay was evaluated using different strains of HSV-1 that were not used in the determination of the limit of detection (LoD) for the assay. No additional strains were available for HSV-2. Quantified viral material was spiked into negative CSF using a single dilution and assayed in triplicate. The Simplexa™ HSV 1 & 2 Direct assay was able to detect other strains of HSV-1 virus.

Analytical Reactivity with Additional Viral Strains

Viral Strain	Concentration [$TCID_{50}$/mL]	Qualitative Results (#Detected/#Total)		
		HSV-1	HSV-2	DNA IC
HSV-1 KOS	20	3/3	0/3	3/3
HSV-1 F	20	2/3	0/3	3/3
	40	3/3	0/3	3/3
	80	3/3	0/3	3/3

Cross-Reactivity (Analytical Specificity)

The Simplexa™ HSV 1 & 2 Direct assay's analytical specificity was evaluated by testing the ability to exclusively identify HSV-1 and HSV-2 viruses with no cross-reactivity to organisms that are closely related, or cause similar clinical symptoms or may be present in CSF. Fifty one (51) potential cross-reactants were spiked into negative CSF and assayed in triplicate. No cross-reactivity was observed.

No.	Cross-Reactant	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	Baseline	Not Applicable	0/20	0/20
2	Adenovirus (type 1)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
3	Adenovirus (type 7A)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
4	BKV	1.00×10^5 copies/mL	0/3	0/3
5	*Citrobacter freundii*	1.00×10^6 cfu/mL	0/3	0/3
6	*Citrobacter koseri*	1.00×10^6 cfu/mL	0/3	0/3
7	*Cryptococcus neoformans*	1.00×10^6 cfu/mL	0/3	0/3
8	Cytomegalovirus (AD169)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
9	Dengue (Type1)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
10	Encephalomyocarditis virus	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
11	Enterobacter aerogenes	1.00×10^6 cfu/mL	0/3	0/3
12	Enterovirus 71	1.00×10^5 $TCID_{50}$/mL	0/3	0/3

No.	Cross-Reactant	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
13	Epstein Barr virus (B95-8)	1.00 X 10^5 copies/mL	0/3	0/3
14	*Escherichia coli*	1.00 X 10^6 cfu/mL	0/3	0/3
15	*Haemophilus influenza*	1.00 X 10^6 cfu/mL	0/3	0/3
16	*Haemophilus influenza*e type b (MinnA)	1.00 X 10^6 cfu/mL	0/3	0/3
17	Hepatitis B	1.00 X 10^5 IU/mL	0/3	0/3
18	Hepatitis C	1.00 X 10^5 IU/mL	0/3	0/3
19	HIV1 (type IIIB)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
20	HIV2 (type NIHZ)	Not Available*	0/3	0/3
21	Human herpesvirus 6	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
22	Human herpesvirus 7 (Type SB)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
23	Human herpesvirus 8	1.00 X 10^5 copies/mL	0/3	0/3
24	Influenza A/California/7/2009 NYMC x-179-A	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
25	Influenza B/Florida/02/2006	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
26	JCV (MAD-4)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
27	*Klebsiella pneumonia*	1.00 X 10^6 cfu/mL	0/3	0/3
28	La Crosse encephalitis	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
29	*Listeria monocytogenes*	1.00 X 10^6 cfu/mL	0/3	0/3
30	Measles	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
31	Mumps	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
32	*Mycobacterium tuberculosis* (Genomic DNA)	1.00 X 10^6 cfu/mL	0/3	0/3
33	*Naegleria fowleri*	1.00 X 10^4 cells/mL	0/3	0/3
34	*Neisseria meningitides* (serogroup A)	1.00 X 10^6 cfu/mL	0/3	0/3
35	Parainfluenza Virus 1	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
36	Parainfluenza Virus 2	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
37	Parainfluenza Virus 3	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
38	Parvovirus B19	1.00 X 10^5 IU/mL	0/3	0/3
39	Poliovirus (Type 3)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
40	*Proteus mirabilis* (Z050)	1.00 X 10^6 cfu/mL	0/3	0/3
41	*Pseudomonas aeruginosa*	1.00 X 10^6 copies/mL	0/3	0/3
42	Rabies	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
43	Rhinovirus (Type 1A)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
44	Rotavirus (Type Wa)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
45	Rubella	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
46	St. Louis Encephalitis	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
47	*Staphylococcus aureus* COL	1.00 X 10^6 cfu/mL	0/3	0/3
48	*Streptococcus agalactiae*	1.00 X 10^6 cfu/mL	0/3	0/3
49	*Streptococcus pneumoniae* Z022; 19F	1.00 X 10^6 cfu/mL	0/3	0/3
50	*Toxoplasma gondii*	1.00 X 10^6 tachyzoites/mL	0/3	0/3
51	Varicella zoster virus	1.00 X 10^5 copies/mL	0/3	0/3
52	West Nile Virus	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3

*Quantified material was not available to test; instead the vendor provided a culture fluid with a known Ct value. The site was directed to dilute the stock to a relevant Ct value; 1:200 dilution factor.

INTERFERENCE

The performance of the Simplexa™ HSV 1 & 2 Direct assay was evaluated with potentially interfering substances that may be present in CSF samples at the concentrations indicated in the table below. A total of seven (7) potentially interfering substances were tested in a low positive HSV-1 and HSV-2 sample (4 times LoD) in CSF matrix and assayed in triplicate. No interference was observed.

Potential Interferent	Interferent Concentration	Qualitative Result (#Detected/#Total)		
		HSV-1	HSV-2	DNA IC
Albumin (protein)	10 mg/mL	3/3	3/3	3/3
Casein (protein)	10 mg/mL	3/3	3/3	3/3
Hemoglobin	0.625 mg/mL	3/3	3/3	3/3
White Blood Cells	5.5×10^8 WBC/mL	3/3	3/3	3/3
Antiviral Drug (Acyclovir)	2.5 mg/mL	3/3	3/3	3/3
Topical Antiseptic (Betadine)	5% (v/v)	3/3	3/3	3/3
Whole Blood	10% (v/v)	3/3	3/3	3/3

COMPETITIVE INTERFERENCE

Competitive interference was studied to evaluate the effects of clinically relevant co-infections with each of the analytes detected by the Simplexa™ HSV 1 & 2 Direct assay. The study assessed whether a high concentration of one virus in the sample could potentially affect the Simplexa™ HSV 1 & 2 Direct assay performance for another target present at low levels. A low sample was contrived at approximately 4 times LoD for each target (HSV-1 McIntryre strain and HSV-2 G strain), and a baseline Ct was determined for each sample. Each potential concomitant infecting virus was spiked into the low level sample and assayed in triplicate. Competitive interference was observed in samples with a very high concentration of HSV-1 virus and a low concentration of HSV-2 virus. Baseline sample results are also shown below.

Baseline (Low Concentration)		Competitive Interferent (High Concentration)		Qualitative Results (#Detected/#Total)		
Strain	Concentration [$TCID_{50}$/mL]	Strain	Concentration [$TCID_{50}$/mL]	HSV-1	HSV-2	DNA IC
HSV-1 McIntyre	20	-	-	5/5	0/5	5/5
HSV-1 McIntyre	20	HSV-2 G	10000	3/3	3/3	3/3
HSV-2 G	5	-	-	0/5	5/5	5/5
HSV-2 G	5	HSV-1 McIntyre	20000	8/8	1/8	8/8
HSV-2 G	5	HSV-1 McIntyre	10000	8/8	6/8	8/8
HSV-2 G	5	HSV-1 McIntyre	5000	3/3	3/3	3/3

INHIBITION BY OTHER MICROORGANISMS

The Simplexa™ HSV 1 & 2 Direct assay was evaluated by testing the ability to identify HSV-1 and HSV-2 viruses when other potentially inhibitory organisms are present. The panel of fifty one (51) potentially inhibitory organisms was individually spiked into a pool with a low concentration (approximately 4 times LoD) of HSV-1 and HSV-2 in CSF. Each microorganism sample was initially tested in triplicate and if any one of the replicates was “Not Detected” for either the HSV-1 or the HSV-2 targets then five additional replicates were tested to confirm if any inhibition was caused by the microorganism. If the majority (>4/8) replicates were “Not Detected” then an inhibitory effect would be determined. None of the microorganisms caused >4/8 of the replicates to be “Not Detected”. 1/8 replicates of JCV (MAD-4) and Rabies were “Not Detected” for HSV-2 and 2/8 replicates of Dengue (Type1) were “Not Detected” for HSV-2. No inhibition by other organisms was observed for either HSV-1 or HSV-2.

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	Baseline	Not Applicable	20/20	20/20
2	Adenovirus (type 1)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
3	Adenovirus (type 7A)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
4	BKV	1.00 X 10^5 copies/mL	3/3	3/3
5	*Citrobacter freundii*	1.00 X 10^6 cfu/mL	3/3	3/3
6	*Citrobacter koseri*	1.00 X 10^6 cfu/mL	3/3	3/3
7	*Cryptococcus neoformans*	1.00 X 10^6 cfu/mL	3/3	3/3
8	Cytomegalovirus (AD169)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
9	Dengue (Type1)	1.00 X 10^5 $TCID_{50}$/mL	8/8	**6/8
10	Encephalomyocarditis virus	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
11	Enterobacter aerogenes	1.00 X 10^6 cfu/mL	3/3	3/3
12	Enterovirus 71	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
13	Epstein Barr virus (B95-8)	1.00 X 10^5 copies/mL	3/3	3/3
14	*Escherichia coli*	1.00 X 10^6 cfu/mL	3/3	3/3
15	*Haemophilus influenzae*	1.00 X 10^6 cfu/mL	3/3	3/3
16	*Haemophilus influenzae* type b (MinnA)	1.00 X 10^6 cfu/mL	3/3	3/3
17	Hepatitis B	1.00 X 10^5 IU/mL	3/3	3/3
18	Hepatitis C	1.00 X 10^5 IU/mL	3/3	3/3
19	HIV1 (type IIIB)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
20	HIV2 (type NIHZ)	Not Available*	3/3	3/3
21	Human herpesvirus 6	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
22	Human herpesvirus 7 (Type SB)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
23	Human herpesvirus 8	1.00 X 10^5 copies/mL	3/3	3/3
24	Influenza A/California/7/2009 NYMC x-179-A	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
25	Influenza B/Florida/02/2006	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
26	JCV (MAD-4)	1.00 X 10^5 $TCID_{50}$/mL	8/8	***7/8
27	*Klebsiella pneumoniae*	1.00 X 10^6 cfu/mL	3/3	3/3
28	LA Crosse encephalitis	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
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32	*Mycobacterium tuberculosis* (Genomic DNA)	1.00 X 10^6 cfu/mL	3/3	3/3
33	*Naegleria fowleri*	1.00 X 10^4 cells/mL	3/3	3/3
34	*Neisseria meningitides* (serogroup A)	1.00 X 10^6 cfu/mL	3/3	3/3
35	Parainfluenza Virus 1	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
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37	Parainfluenza Virus 3	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
38	Parvovirus B19	1.00 X 10^5 IU/mL	3/3	3/3
39	Poliovirus (Type 3)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
40	*Proteus mirabilis* (Z050)	1.00 X 10^6 cfu/mL	3/3	3/3
41	*Pseudomonas aeruginosa*	1.00 X 10^6 copies/mL	3/3	3/3

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
42	Rabies	1.00 X 10^5 $TCID_{50}$/mL	8/8	***7/8
43	Rhinovirus (Type 1A)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
44	Rotavirus (Type Wa)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
45	Rubella	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
46	St. Louis Encephalitis	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
47	*Staphylococcus aureus* COL	1.00 X 10^6 cfu/mL	3/3	3/3
48	*Streptococcus agalactiae*	1.00 X 10^6 cfu/mL	3/3	3/3
49	*Streptococcus pneumoniae* Z022; 19F	1.00 X 10^6 cfu/mL	3/3	3/3
50	*Toxoplasma gondii*	1.00 X 10^6 tachyzoites/mL	3/3	3/3
51	Varicella zoster virus	1.00 X 10^5 copies/mL	3/3	3/3
52	West Nile Virus	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3

*Quantified material was not available to test; instead the vendor provided a culture fluid with a known Ct value. The site was directed to dilute the stock to a relevant Ct value; 1:200 dilution factor.
**1/3 and 1/5 replicates were "Not Detected" for HSV-2 during initial and confirmatory testing respectively.
***1/3 replicate was "Not Detected" for HSV-2 during initial testing.

CARRY-OVER CONTAMINATION

The amplification carry-over for the Simplexa™ assays including the Simplexa™ HSV 1 & 2 Direct assay was assessed from Simplexa™ Flu A/B & RSV Direct REF MOL2650 (K120413) assay and can be found on the FDA website. The study can be applied to the Simplexa™ HSV 1 & 2 Direct assays as the study is not analyte specific. In the Simplexa™ Flu A/B & RSV Direct REF MOL2650 (K120413), the amplification carry-over study searched for the presence of contamination in negative samples. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was seen.

FRESH VS FROZEN STUDY

Samples should be transported on ice and stored at 2 to 8°C for up to 7 days post collection. If there is a greater than 7 day delay before processing of the sample, store the sample at -70° C.

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