EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Simplexa™ HSV 1 & 2 Direct

DECISION SUMMARY

A. 510(k) Number:

K133621

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the Simplexa™ HSV 1 & 2 Direct

C. Measurand:

Target DNA sequences from conserved regions of the HSV-1 and HSV-2 DNA polymerase genes.

D. Type of Test:

A real-time Polymerase Chain Reaction (PCR) for the direct amplification, detection and differentiation of HSV-1 and HSV-2 DNA from unprocessed CSF samples without nucleic acid extraction.

E. Applicant:

Focus Diagnostics, Inc.

F. Proprietary and Established Names:

Simplexa™ HSV 1 & 2 Direct

G. Regulatory Information:

1. Regulation: 21 CFR 866.3307
2. Classification: Class II (special controls)
3. Product code: PGH
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

   Simplexa™ HSV 1 & 2 Direct
   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.

   The Positive Control is intended to be used as a control with the Simplexa™ HSV 1 & 2 Direct. This control is not intended for use with other assays or systems.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   The Simplexa™ HSV 1 & 2 Direct is for prescription use only in accordance with 21 CFR 801.109.

4. Special instrument requirements:

   To be used with the 3M™ Integrated Cycler with Integrated Cycler Studio Software version 5.0 or higher

I. Device Description:

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

In the Simplexa™ HSV 1 & 2 Direct, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify HSV-1, HSV-2 and DNA internal control (IC) 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. The IC

is used to detect PCR failure and/or inhibition.

The 3M Integrated Cycler is a real-time PCR thermocycler which uses real-time fluorometric detection to identify targets within the sample wells. The instrument is controlled by an external computer running the Integrated Cycler Studio Software.

The Simplexa™ HSV 1 & 2 Direct assay reaction takes place in the DAD consumable. The DAD consumable is compartmentalized into 8 separate wedges and up to 8 separate samples or controls may be processed on each disc. Each wedge can be used only once, however, the disc may be reused until all wedges have been utilized. Each wedge contains sample and reagent input wells, microfluidic channels and laser activated valves to control the fluid flow, and a reaction chamber. The disc is specifically designed to meter the amount of reagent (Reaction Mix) and sample that are placed into specific wells in the disc. To start processing a patient sample, a foil seal is lifted and the user adds 50 µL of Reaction Mix to the reagent input well using a fixed volume pipette. Next, the user adds 50 µL of unextracted specimen to the sample input well.

The Simplexa™ HSV 1 & 2 Direct kit contains reagents for 24 reactions. Each vial contains sufficient material for a single reaction. The kit contains the Reaction Mix, the Simplexa™ HSV 1 & 2 Direct Barcode Card with assay specific information, and the Package Insert.

Component Description

<table>
<tr><th>Kit Component</th><th>Contents</th></tr>
<tr><td>Simplexa™ HSV 1 & 2 Direct Reaction Mix</td><td>
<table>
<tr><td colspan="5">DNA polymerase, buffer, dNTPs, template DNA (IC), dye-labeled fluorescent probe-primers specific for detection of HSV-1, HSV-2, and the DNA IC.</td></tr>
<tr><th>Target</th><th>Probe Fluorophore (Dye)</th><th>Excitation (nm)</th><th>Emission (nm)</th><th>Targeted Gene</th></tr>
<tr><td>HSV-1</td><td>CFR610</td><td>590</td><td>610</td><td>HSV-1 DNA polymerase</td></tr>
<tr><td>HSV-2</td><td>FAM</td><td>495</td><td>520</td><td>HSV-2 DNA polymerase</td></tr>
<tr><td>DNA IC</td><td>Q670</td><td>644</td><td>670</td><td>NA</td></tr>
</table>
</td></tr>
<tr><td>Simplexa™ HSV 1 & 2 Kit Barcode Card</td><td>Assay specific parameters.</td></tr>
</table>

The Positive Control contains ten vials of Inactivated HSV-1 and HSV-2.

J. Standard/Guidance Documents Referenced:

Guidance for Industry and FDA Staff: Administrative Procedures for CLIA Categorization, May 7, 2008

Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k), August 12, 2005

Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices, September 9, 1999

Guidance for Industry: Cybersecurity for Networked Medical Devices Containing Off-The-Shelf (OTS) Software, July 18, 2007

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, May 11, 2005

General Principles of Software Validation: Final Guidance for Industry and FDA Staff, January 11, 2002

K. Test Principle:

The Simplexa™ HSV 1 & 2 Direct system is a real-time PCR for the direct amplification, detection and differentiation of HSV-1 and HSV-2 DNA from unprocessed CSF specimens without nucleic acid extraction. The system consists of the Simplexa™ HSV 1 & 2 Direct, 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 reaction, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify HSV-1, HSV-2 and IC 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. The principle of the test and the procedural steps are summarized below.

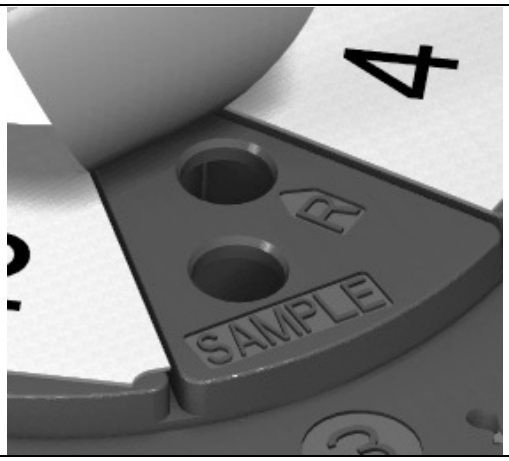
Specimen Collection:
The specimen type is CSF. Specimens should be transported on ice and stored at 2 to 8°C for up to 7 days post collection. If there is delay for more than 7 days before processing, the specimens should be stored at -70°C.

Real-Time PCR Instrument Setup:
The 3M Integrated Cycler Operator Manual provides details on how to configure the 3M Integrated Cycler Studio Software, to add an assay definition, and to set up and analyze runs on the 3M Integrated Cycler.

Direct Amplification Disc Loading and Real-Time PCR Amplification:

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 (Figures 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 the Reaction (R) well.
9. Use the fixed volume pipette to transfer 50 µL of sample or control; pipette sample or control into the 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 Direct Amplification Disc 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**
	

Interpretation of Results:

Upon completion of the run, the Integrated Cycler Studio Software automatically calculates and displays results. The display presents the user with a separate report box for each of the analytes as well as the IC. The IC can be reported as “valid” or “invalid”.

The software reports one of four possible outcomes for each of HSV-1 and HSV-2 after a run

is completed for each sample ID entered: “Detected”, “Not Detected”, “Invalid” or “EC500”.

a. “Detected” result indicates that HSV-1 and/or HSV-2 DNA were detected in the patient sample tested (whether the DNA IC was detected or not detected)

b. “Not Detected” result indicates that HSV-1 and/or HSV-2 DNA were not detected in the patient sample tested whereas the DNA IC was detected.

c. “Invalid” result points to the inability to detect 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.

    If an invalid result occurs, the sample needs to be re-tested per the instructions provided in the device Package Insert.

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.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Reproducibility

The reproducibility study evaluated the device’s inter-laboratory, inter-assay, and intra-assay reproducibility for high negative, low positive (approximately equal to the Limit of Detection (LoD)) and moderately positive (approximately 4 times LoD) samples for HSV-1 (McIntyre strain) and HSV-2 (G strain) and a positive control. The testing panel consisted of a total of 6 samples: a high negative pool containing both HSV-1 and HSV-2 targets, a low positive and a moderately positive sample separately for each of HSV-1 and HSV-2, and a positive control containing both HSV-1 and HSV-2 targets. The samples (except for the positive control) were generated by spiking viral stock into CSF that was screened to be negative for both analytes. For each sample panel member, two different operators performed two runs per day with 3 replicates per run, for five days at each of the three sites. This provided a total of 90 (3 sites x 5 days x 2 runs (each by different operator) x 3 replicates) replicates per sample panel member over five non-consecutive days. Three sites assessed the device's inter-laboratory reproducibility and inter/intra-assay reproducibility. Combined results for all sites and results stratified by site are presented in the tables below.

Reproducibility – 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-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 for HSV-2 Low Positive, HSV-2 Medium Positive and High Negative samples are "Negative" for HSV-1.

Reproducibility – 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		
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) [b]	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 for HSV-1 Low Positive, HSV-1 Medium Positive and High Negative samples are "Negative" for HSV-2.

Reproducibility – DNA IC

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 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%**

The positive control Inter-Lot precision was evaluated to assess if there was lot to lot

variability of the positive control. The study assessed the reproducibility of 3 different lots of positive control Pack by testing each lot of control with one lot of Reaction Mix, using one instrument and one operator over 3 consecutive days. Each lot of positive control was run with 2 replicates per run, 2 runs per day for 3 days, thereby providing a total 12 replicates per lot. The testing was performed at Focus Diagnostics. Results of the study were analyzed for overall mean, overall (total) variability, variability between day (inter-day), variability between run (inter-run), variability between lot (inter-lot) and the residual. The variability is presented in terms of standard deviation (SD) and/or percent coefficient of variation (%CV) of Ct. The inter-lot variability component is summarized in the table below.

Quantitative Summary of PC Inter-Lot Reproducibility												
			Inter-Day		Inter-Run		Inter-Lot		Intra-Run/Lot		Total	
Analyte	**N**	**Mean Ct**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
HSV-1	36	29.4	0.00	0.0	0.00	0.0	0.11	0.4	0.11	0.4	0.16	0.5
HSV-2	36	29.8	0.00	0.0	0.00	0.0	0.12	0.4	0.13	0.5	0.18	0.6
IC	36	29.6	0.00	0.0	0.08	0.3	0.08	0.3	0.19	0.7	0.22	0.8

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Positive Control Stability:
The positive control for this product is a blend of inactivated HSV-1 and HSV-2 provided in single use aliquots frozen at -20°C. The positive control has been assigned an expiration date of 12 months at -20°C.
To assess the room temperature stability of positive control, aliquots of the positive control were thawed and kept at room temperature for up to 24 hours, and were then evaluated.

Room-temperature stability of Positive Control

	HSV-2 (FAM)		HSV-1 (CFR610)		IC (Q670)	
Time (hrs) at room temperature	**Mean Ct**	**Change from time '0'**	**Mean Ct**	**Change from time '0'**	**Mean Ct**	**Change from time '0'**
0	30.6	-0.33%	30.7	0.65%	30.1	0.66%
8 hours	30.5		30.9		30.3	
0	30.8	0.00%	31.3	-0.96%	30.2	0.00%
24 hours	30.8		31.0		30.2	

A negative percent difference indicates earlier Ct detection after room temperature storage. As shown in the table above, no significant change in detection of each target analyte was observed when positive control was stored on the bench top at room temperature for 24 hours. Based on these results, the positive control is stable for at least 24 hours at room temperature.

d. Assay cut-off:

The Simplexa™ HSV 1 & 2 Direct cut-off was determined during feasibility and verification studies. During verification, all studies were performed to a maximum of 45 cycles of amplification for informational use, but the data were calculated with a cut-off of Ct=40 for HSV-1 and HSV-2 targets. When the verification data were analyzed, there were some results that prompted the Ct cut-off for HSV-2 to be re-set from Ct=40 to Ct=42. The LoD verification study detected HSV-2 between a Ct of 40 and 41 for four replicates at HSV-2 concentrations below the established HSV-2 LoD concentration. No HSV-2 LoD replicates (at or below LoD concentration) were detected beyond Ct=41. The HSV-1 cut-off was not adjusted from Ct=40 because no HSV-1 LoD replicates from the LoD verification study were detected at a Ct>40. Based on the results of the verification studies, the final cut-offs were set at Ct=40 for HSV-1 and at Ct=42 for HSV-2. The PCR cycling protocol was reduced to 42 cycles because all target cut-offs are at Ct≤42. The Ct cut-offs and cycling protocol is stored in the assay definition for the Simplexa™ HSV 1 & 2 Direct.

e. Detection limit (LoD)

The Limit of Detection (LoD) was determined for the Simplexa™ HSV 1 & 2 Direct using viral stocks of HSV-1 and HSV-2. The samples used for the study were prepared using a total of four strains of HSV-1 (McIntyre and HF strains) and HSV-2 (G and MS strains) that were individually diluted into bulk negative human CSF matrix. Each strain of HSV used in the study consisted of verified stock material that had been regrown and retitered from the original source. The study consisted of multiple runs for each strain to evaluate the LoD of Simplexa™ HSV 1 & 2 Direct and all samples were stored overnight at -20°C or below prior to LoD testing.

For each of the HSV-1 McIntyre and HSV-2 G strains, screening and confirmation testing was consolidated into 32 DAD runs using two lots of Simplexa™ HSV 1 & 2 Direct across four instruments. For each of the two targets, the designated strains were spiked into negative human CSF matrix and serially diluted into 2-fold serial dilutions to encompass concentrations around the theoretical LoD. In total, each dilution was assayed in 32 replicates across the 32 DAD runs, where each run consisted of one replicate of each dilution. Per target strain, each instrument underwent eight runs in total, where four of the runs used material from one designated Simplexa™ HSV 1 & 2 Direct lot and the other four runs used material from a second designated Simplexa™ HSV 1 & 2 lot, resulting in 32 runs per strain.

The LoDs for the remaining two strains (HSV-1 HF strain and HSV-2 MS strain) were determined from screening runs that provided tentative LoDs for both the HSV-1 and HSV-2 strains and confirmation runs to confirm the respective LoDs. To determine the tentative LoDs, the designated strains were spiked into negative human CSF matrix and serially diluted to their approximate theoretical LoDs. For each of these two strains, at least three replicates of each dilution were tested and the lowest concentration at which all replicates were detected was taken as the tentative LoD. For the confirmation of tentative LoD, each designated strain was spiked into negative human CSF matrix at the concentration of the tentative LoD. The samples for each strain were prepared in singlicate and assayed in 32 replicates across multiple disc runs using a single lot of Simplexa™ HSV 1 & 2 Direct. The LoD was determined as the lowest concentration for which ≥ 95% of the replicates were detected.

Limit of Detection Summary

Virus Strain	LoD ($TCID_{50}$/mL)	Qualitative Results at LoD (#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

f. Analytical Reactivity

The analytical reactivity of the Simplexa™ HSV 1 & 2 Direct was evaluated using different strains of HSV-1 that were not used to determine the 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 was able to detect both strains of HSV-1 virus at 20 $TCID_{50}$/mL.

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

g. Analytical specificity:

Cross-Reactivity:

The analytical specificity of the Simplexa™ HSV 1 & 2 Direct was evaluated by testing 51 potential cross-reactants that are closely related, cause similar clinical symptoms, or may be present in CSF. The potential cross-reactants were spiked into negative CSF and assayed in triplicate. No cross-reactivity was observed as summarized in the following table.

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Simplexa™ HSV 1 & 2 Direct Cross-Reactivity Results

Cross-Reactant	Tested Concentration	Qualitative Result (#Detected/#Total)	
		HSV-1	HSV-2
Baseline	Not Applicable	0/20	0/20
Adenovirus (type 1)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
Adenovirus (type 7A)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
BKV	1.00×10^5 copies/mL	0/3	0/3
Citrobacter freundii	1.00×10^6 cfu/mL	0/3	0/3
Citrobacter koseri	1.00×10^6 cfu/mL	0/3	0/3
Cryptococcus neoformans	1.00×10^6 cfu/mL	0/3	0/3
Cytomegalovirus (AD169)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
Dengue (Type1)	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
Encephalomyocarditis virus	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
Enterobacter aerogenes	1.00×10^6 cfu/mL	0/3	0/3
Enterovirus 71	1.00×10^5 $TCID_{50}$/mL	0/3	0/3
Epstein Barr virus (B95-8)	1.00×10^5 copies/mL	0/3	0/3

Cross-Reactant	Tested Concentration	Qualitative Result (#Detected/#Total)	
		HSV-1	HSV-2
Escherichia coli	1.00 X 10^6 cfu/mL	0/3	0/3
Haemophilus influenzae	1.00 X 10^6 cfu/mL	0/3	0/3
*Haemophilus influenza*e type b (MinnA)	1.00 X 10^6 cfu/mL	0/3	0/3
Hepatitis B	1.00 X 10^5 IU/mL	0/3	0/3
Hepatitis C	1.00 X 10^5 IU/mL	0/3	0/3
HIV1 (type IIIB)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
HIV2 (type NIHZ)	Not Available*	0/3	0/3
Human herpesvirus 6	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Human herpesvirus 7 (Type SB)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Human herpesvirus 8	1.00 X 10^5 copies/mL	0/3	0/3
Influenza A/California/7/2009 NYMC x-179-A	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Influenza B/Florida/02/2006	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
JCV (MAD-4)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Klebsiella pneumoniae	1.00 X 10^6 cfu/mL	0/3	0/3
La Crosse encephalitis	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Listeria monocytogenes	1.00 X 10^6 cfu/mL	0/3	0/3
Measles	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Mumps	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Mycobacterium tuberculosis (Genomic DNA)	1.00 X 10^6 cfu/mL	0/3	0/3
Naegleria fowleri	1.00 X 10^4 cells/mL	0/3	0/3
Neisseria meningitides (serogroup A)	1.00 X 10^6 cfu/mL	0/3	0/3
Parainfluenza Virus 1	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Parainfluenza Virus 2	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Parainfluenza Virus 3	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Parvovirus B19	1.00 X 10^5 IU/mL	0/3	0/3
Poliovirus (Type 3)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Proteus mirabilis (Z050)	1.00 X 10^6 cfu/mL	0/3	0/3

Cross-Reactant	Tested Concentration	Qualitative Result (#Detected/#Total)	
		HSV-1	HSV-2
Pseudomonas aeruginosa	1.00 X 10^6 copies/mL	0/3	0/3
Rabies	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Rhinovirus (Type 1A)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Rotavirus (Type Wa)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Rubella	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
St. Louis Encephalitis	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
Staphylococcus aureus COL	1.00 X 10^6 cfu/mL	0/3	0/3
Streptococcus agalactiae	1.00 X 10^6 cfu/mL	0/3	0/3
Streptococcus pneumoniae Z022; 19F	1.00 X 10^6 cfu/mL	0/3	0/3
Toxoplasma gondii	1.00 X 10^6 tachyzoites/mL	0/3	0/3
Varicella zoster virus	1.00 X 10^5 copies/mL	0/3	0/3
West Nile Virus	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3

h. Interference studies:

Interfering Substances:

The effect of potentially interfering substances that may be present in CSF specimens was evaluated at the concentrations indicated in the table below. A total of 7 potentially interfering substances was 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 x 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. The study assessed whether a high concentration of one virus in the specimen could potentially affect the Simplexa™ HSV 1 & 2 Direct performance for the other viral target present at low levels. A low level 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 specimen 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.

Simplexa™ HSV 1 & 2 Direct Competitive Interference Results

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 was evaluated by testing the ability to identify HSV-1 and HSV-2 viruses when other potentially inhibitory organisms are present. The panel of 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". One of 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.

Simplexa™ HSV 1 & 2: Inhibition by Other Microorganisms

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

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
28	LA Crosse encephalitis	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
29	*Listeria monocytogenes*	1.00 X 10^6 cfu/mL	3/3	3/3
30	Measles	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
31	Mumps	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
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
36	Parainfluenza Virus 2	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
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
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.

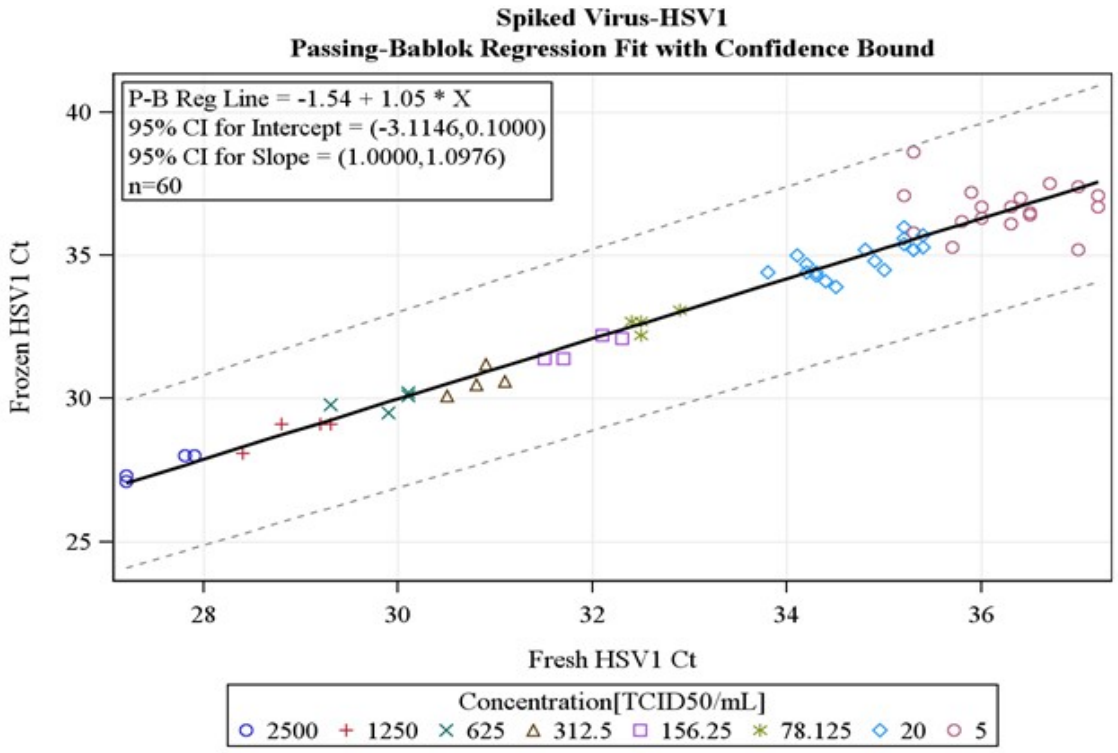
i. Sample stability studies

The objective of this study was to evaluate the difference between results from the Simplexa™ HSV 1 & 2 Direct testing of fresh (never frozen, unextracted and tested within 72 hours of preparation) specimens versus frozen (unextracted and tested after

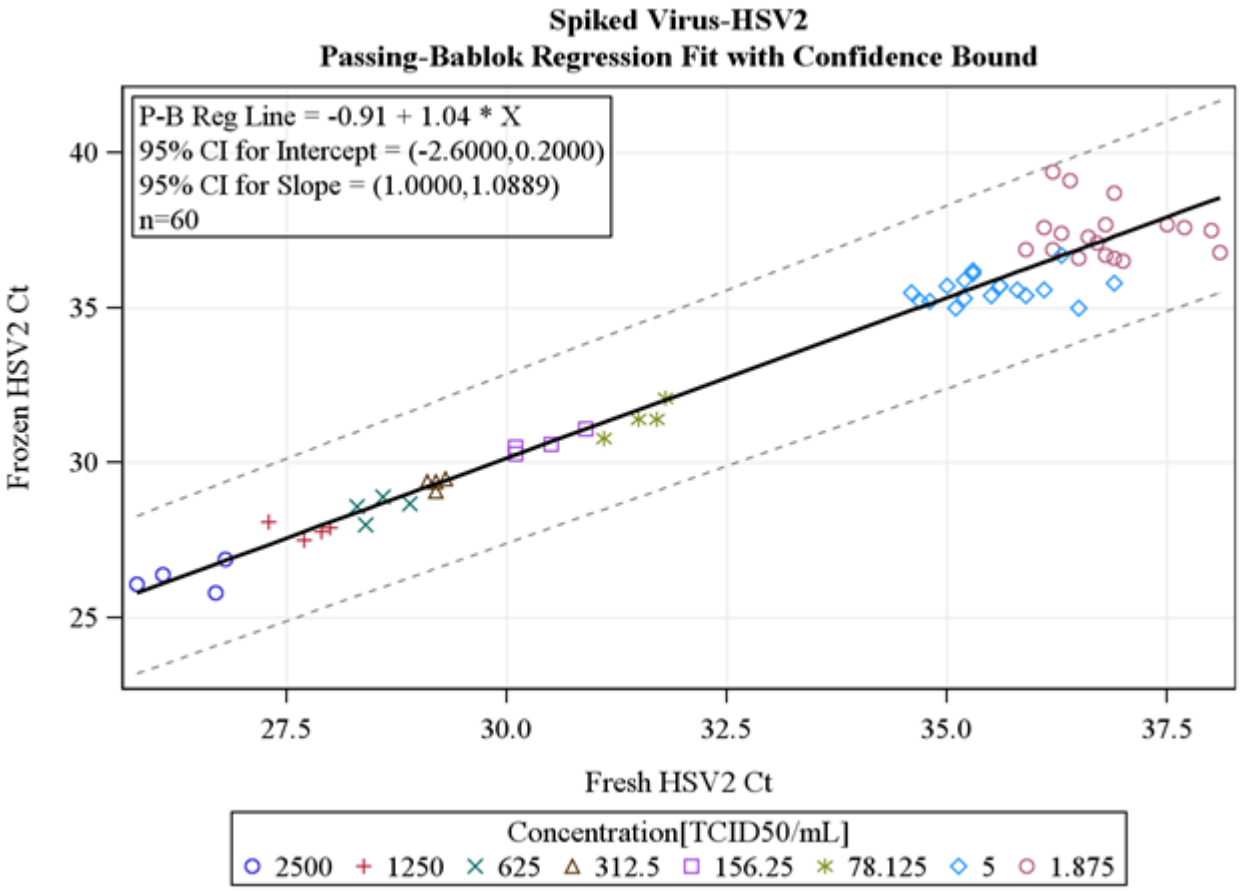
2 freeze-thaw steps) specimens. A panel of contrived samples consisting of 120 pairs (fresh and frozen) of spiked specimens prepared in negative human CSF matrix was used for this study; 60 of the pairs spanned the analytical range for HSV-1 and the other 60 pairs spanned the range for HSV-2. Sixty percent of the samples (36/60) were spiked to be close to LoD so that 18 of the samples were 1-1.5 times LoD and 18 samples were spiked at 4 times LoD. The remaining 40% of the samples (24/60) were spiked across the assay range. One lot of Simplexa ™ HSV 1 & 2 Direct was used for the study. The frozen samples were stored at -20°C for a minimum of 5 days, after which they were thawed for at least 1 hour and refrozen for >24 hours and then thawed again for testing. The HSV-1 (McIntyre) and HSV-2 G strains were used in the study.

No significant storage effect on the samples stored fresh was detected when compared to the results from the same samples frozen. Below is the summary of results from the Fresh vs. Frozen Study of the Simplexa™ HSV 1 & 2 Direct along with the concentration [TCID50/mL] of the virus strains. In the Simplexa™ HSV 1 & 2 Direct Fresh vs. Frozen study, 100% (60/60) qualitative agreement was observed with 95% CI (94.0% to 100.0%) between the fresh and frozen states for both HSV-1 and HSV-2 targets. Regression analysis was performed to estimate quantitative bias (in terms of Ct) between Ct values from the paired fresh and frozen samples. The regression plots are shown below. The 95% confidence intervals for intercept and slope of both targets indicate statistically non-significant bias between Ct values for fresh and frozen samples. These results support the testing of frozen samples to establish performance characteristics of the assay.

HSV-1 Sample Distribution	
HSV-1 (McIntyre) Spiked Concentration [$TCID_{50}$/mL]	Number of Replicates (% of Total)
5 (LoD)	18 (30%)
20 (4XLoD)	18 (30%)
78.125	4 (6.7%)
156.25	4 (6.7%)
312.5	4 (6.7%)
625	4 (6.7%)
1250	4 (6.7%)
2500	4 (6.7%)
Total	**60 (100%)**



HSV-2 Sample Distribution	
HSV-2 (G strain) Spiked Concentration [TCID50/ mL]	Number of Replicates (% of Total)
1.875 (1.5XLoD)	18 (30%)
5 (4XLoD)	18 (30%)
78.125	4 (6.7%)
156.25	4 (6.7%)
312.5	4 (6.7%)
625	4 (6.7%)
1250	4 (6.7%)
2500	4 (6.7%)
Total	**60 (100%)**



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j. Stability studies

A real-time stability was completed over a 14 month time period supporting a shelf-life claim of 12 months for the Simplexa™ HSV 1 & 2 Direct and the positive control.

k. Carry-over Contamination

The amplification carry-over for the Simplexa™ assays including the DirectSimplexa™ HSV 1 & 2 Direct Simplexa™ HSV 1 & 2 Direct was assessed from the Simplexa™ Flu A/B & RSV Direct (K120413). The carry-over study in K120413 can be applied to the Simplexa™ HSV 1 & 2 Direct as carry-over contamination is not expected to be analyte specific and there have been no hardware changes to the instrument since the K120413 clearance. The carry-over study in K120413 searched for the presence of contamination in negative samples that were adjacent to high positive samples. The study was designed by alternately placing high positive and negative samples on each disc. A total of 60 negative samples and 60 high positive samples were tested across 17 runs using 4 different instruments. The

sample size of 60 was chosen to provide an acceptance criterion of > 90% for the lower bound of the two-sided 95% Confidence Interval for the observed negative rate of the negative samples. This acceptance criterion would be met if no more than one of the 60 negative samples gave a "Detected" result indicative of carry-over contamination. All 60 negative samples gave a result of "Not Detected" for all three target viruses in the K120413 study with a negative rate of 100% and a lower bound of the two-sided 95% CI of 94.0%. Therefore, no evidence of carryover was observed.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies section of this document.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

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 at 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 for testing with the Simplexa™ HSV 1 & 2 Direct. Five external sites, which included four of the collection sites, performed testing with the Simplexa™ HSV 1 & 2 Direct. The other aliquot was retained at Focus Diagnostics for the comparator PCR/sequencing method.

Results from the Simplexa™ HSV 1 & 2 Direct were compared to results from two PCR/bi-directional sequencing assays (comparator). A positive result by the comparator is defined as a positive by at least one of the two PCR/bidirectional sequencing assays. For a result to be defined as negative by the comparator, both of the two PCR/bidirectional sequencing assays should yield negative results. The samples were tested using two PCR reactions targeting two distinct regions of the HSV genome followed by analysis with two bi-directional sequencing 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.

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 by the Simplexa HSV1&2 Direct and the comparator algorithm. The table below shows the distribution of the different clinical parameters collected from patients who were confirmed positive and negative by the results from the two PCR/bi-directional sequencing assays.

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 the 2 PCR/bidirectional sequencing assays).

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 two PCR/bi-directional sequencing assays. These retrospective samples 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%

4. Expected Values

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

Sample Population	**Sample Collection Site**	**No. of Samples**	**Expected Values Based on Simplexa™ HSV1/2 Direct**		
			HSV-1	**HSV-2**	**Not Detected**
Prospective	1	31	-	6.5% (2/31)	93.5% (29/31)
	7	24	4.2% (1/24)	-	95.8% (23/24)
	8	94	4.3% (4/94)	5.3% (5/94)	90.4% (85/94)
	9	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 Positive by Simplexa™ HSV1/2 Direct											
	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		1	9	10			6	6		1	3	4
>1 month to 2 years		1	25	26			8	8		1	17	18
>2 years to 12 years			9	9			6	6			3	3
>12 years to 21 years		1	11	12		1	4	5			7	7
>21 years to 60 years	4	4	65	73	3	1	35	39	1	3	30	34
>60 years	1		33	34	1		20	21			13	13
All	**5**	**7**	**152**	**164**	**4**	**2**	**79**	**85**	**1**	**5**	**73**	**79**

M. Instrument Names:

Integrated Cycler with Integrated Cycler Studio Software version 5.0 or higher (3M)

N. System Description:

1. Modes of Operation:
   Batch

2. Specimen Identification:
   Entered manually by user

3. Specimen Sampling and Handling:
   Samples are processed according to assay instructions.

4. Calibration:
   In-field calibration for the Integrated Cycler is not necessary. Calibration of the optical modules (excitation and emission gain settings) is performed during the manufacturing process and the values are stored in the instrument firmware.

5. Quality Control:
   Quality control is addressed for each separately cleared specific assay to be run on the instrument.

   The positive control 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 Diagnostics 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 Direct package insert 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.

6. Software:
   FDA has reviewed applicant's Hazard Analysis and Software Development processes for this line of product types:
   Yes___X_____ or No_____

O. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not Applicable

P. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Parts 801 and 809, 21 CFR 801.109, and the special controls.

Q. Identified Potential Risks and Required Mitigation Measures

Identified Potential Risks	**Required Mitigation Measures**
Risk of false results	Special controls (1), (2), and (3)
Failure to correctly interpret test results	Special controls (4) and (5)
Failure to correctly operate the instrument	Special controls (6) and (7)

R. Benefit/Risk Analysis

Summary	
Summary of the Benefit(s)	When used for the proposed intended use, the benefits to the clinician and the patient include: (1) establishment of the device performance in a manner that demonstrates consistent accurate test results; and 2) ability to use a well validated device to diagnose HSV in the CNS, which will allow prompt initiation of disease specific treatment.

Summary of the Risk(s)	The risks associated with the device, when used as intended, are those related to the risk of false results, failure to correctly interpret the test results and failure to correctly operate the instrument. They may lead to error or delay in the diagnosis of HSV-1 and HSV-2 infections, error or delay in treatment of these infections, unnecessary use of anti-viral therapy, and delay in establishing the patient's true diagnosis.
Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of this device outweigh the probable risks associated with its use. There are no substantial clinical concerns with the classification of this device in Class II given the combination of general and special controls.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3307 with special controls. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Device Type: Herpes simplex virus nucleic acid-based assay for central nervous system infections.

Class: II (special controls)

Regulation: 21 CFR 866.3307

(a) *Identification*. A herpes simplex virus nucleic acid-based assay for central nervous system infections is a qualitative *in vitro* diagnostic device intended for detection and differentiation of HSV-1 and HSV-2 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.
(b) *Classification*. Class II (special controls). The special controls for this device are:

1) Premarket notification submissions must include detailed documentation for the device description, including the device components, ancillary reagents required but not provided, and a detailed explanation of the methodology including primer design

and selection.

2) Premarket notification submissions must include detailed documentation from the following analytical and clinical performance studies: Analytical sensitivity (Limit of Detection), reactivity, inclusivity, precision, reproducibility, interference, cross reactivity, carry-over, and cross contamination. Premarket notification submissions must also document reagent and sample stability recommendations.

3) Premarket notification submissions must include detailed documentation from a clinical study. The study, performed on a study population consistent with the intended use population, must compare the device performance to the results of two PCR methods followed by bidirectional sequencing.

4) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.

5) The device labeling must include a limitation statement that reads: “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.”

6) Premarket notification submissions must include quality assurance protocols and a detailed documentation for device software, including, but not limited to, standalone software applications and hardware-based devices that incorporate software.

7) The risk management activities performed as part of the manufacturer’s 21 CFR 820.30 design controls must document an appropriate end user device training program that will be offered as part of efforts to mitigate the risk of failure to correctly operate the instrument.