March 14, 2018

DiaSorin Molecular LLC
Sharon Young
Senior Regulatory Affairs Specialist
11331 Valley View Street
Cypress, California 90630

Re: K173798
Trade/Device Name: Simplexa HSV 1 & 2 Direct, Simplexa HSV 1 & 2 Positive Control Pack
Regulation Number: 21 CFR 866.3309
Regulation Name: Herpes Virus Nucleic Acid-Based Cutaneous and Mucocutaneous Lesion Panel
Regulatory Class: Class II
Product Code: PGI
Dated: December 12, 2017
Received: December 14, 2017

Dear Sharon Young:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR

Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Steven R. Gitterman -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*

Device Name
Simplexa™ HSV 1 & 2 Direct MOL2150 and Simplexa™ HSV 1 & 2 Positive Control Pack MOL2160

Indications for Use *(Describe)*

Simplexa™ HSV 1 & 2 Direct MOL2150

The DiaSorin Molecular Simplexa™ HSV 1 & 2 Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA present in mucocutaneous and cutaneous lesion swabs from patients with signs and symptoms of HSV-1 or HSV-2 infection. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 infections.

The assay is not intended for use as a screening test for the presence of HSV-1 and HSV-2 in blood or blood products. The assay is for professional use only.

Simplexa™ HSV 1 & 2 Positive Control Pack MOL2160

The Simplexa™ HSV 1 & 2 Positive Control Pack is intended to be used as a control with the Simplexa™ HSV 1 & 2 Direct kit.

This control is not intended for use with other assays or systems.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Applicant	DiaSorin Molecular LLC. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Sharon Young tel 562.240.6680 fax 562.240.6529 Sharon.Young@DiaSorin.com
Summary Date	March 9, 2018
Proprietary Name	Simplexa™ HSV 1 & 2 Direct MOL2150 and Simplexa™ HSV 1 & 2 Positive Control Pack MOL2160
Generic Name	HSV 1 & 2 nucleic acid
Classification Regulation Product Code	PGI
Regulation Number	866.3309
Regulation name	Herpes Virus (VZV, HSV1, HSV2), DNA Detection Assay for Cutaneous and Mucocutaneous Lesion Samples
Predicate Devices	Lyra® Direct HSV 1 + 2/VZV Assay K133448

Intended Use

Simplexa™ HSV 1 & 2 Direct
The DiaSorin Molecular Simplexa™ HSV 1 & 2 Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA present in mucocutaneous and cutaneous lesion swabs from patients with signs and symptoms of HSV-1 or HSV-2 infection. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 infections.

The assay is not intended for use as a screening test for the presence of HSV-1 and HSV-2 in blood or blood products. The assay is for professional use only.

Simplexa™ HSV 1 & 2 Positive Control Pack
The Simplexa™ HSV 1 & 2 Positive Control Pack is intended to be used as a control with the Simplexa™ HSV 1 & 2 Direct kit.

This control is not intended for use with other assays or systems.

Device Description

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 cutaneous and mucocutaneous lesion swab specimens without nucleic acid extraction. The system consists of the Simplexa™ HSV 1 & 2 Direct assay, the LIAISON® MDX (with LIAISON® MDX 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.

Simplexa™ HSV 1 & 2 Direct REF MOL2150

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	C	RM	Brown	24	1/24	50 µL

Component Description

<table>
<tr><th>Kit Component</th><th>Contents</th></tr>
<tr><td>Simplexa™ HSV 1 & 2 Direct Reaction Mix (RM)</td><td>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.
<table>
<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 Internal Control</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>

Simplexa™ HSV 1 & 2 Positive Control Pack REF MOL2160

Component Name	REF	Description	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ HSV 1 & 2 Direct Positive Control	MOL2161	Inactivated HSV 1 and 2 viruses	Red	10	1/10	100 µL

Direct Amplification Disc kit REF MOL1455

Component Name	REF	Description	Number of Discs	Reactions per Disc/Kit
Direct Amplification Disc	MOL1452	Direct Amplification Disc for the processing of up to 8 individual controls or specimens	3	8/24

Predicate Device Information

Table of Similarities		
Name	**Predicate**	**Device**
	Lyra® Direct HSV 1 + 2/VZV Assay K133448	**Simplexa™ HSV 1 & 2 Direct**
Intended Use	The Lyra® Direct HSV 1 + 2/VZV Assay is an in vitro multiplex Real-Time PCR test for qualitative detection and differentiation of herpes simplex virus type 1, herpes simplex virus type 2, and varicella-zoster virus DNA isolated and purified from cutaneous or mucocutaneous lesion samples obtained from symptomatic patients suspected of active herpes simplex virus 1, herpes simplex virus 2 and/or varicella-zoster infection. The Lyra ® Direct HSV 1 + 2/VZV Assay is intended to aid in the diagnosis of herpes simplex virus 1, herpes simplex virus 2 and varicella-zoster virus active cutaneous or mucocutaneous infections. Negative results do not preclude herpes simplex virus 1, herpes simplex virus 2 and varicella-zoster virus infections and should not be used as the sole basis for diagnosis, treatment or other management decisions. The Lyra® Direct HSV 1 + 2/VZV Assay is not intended for use with cerebrospinal fluid or to aid in the diagnosis of HSV or VZV infections of the central nervous system (CNS). The Lyra® Direct HSV 1 + 2/VZV Assay is not intended for use in prenatal screening. The device is not intended for point-of-care use.	The DiaSorin Molecular Simplexa™ HSV 1 & 2 Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA present in mucocutaneous and cutaneous lesion swabs from patients with signs and symptoms of HSV-1 or HSV-2 infection. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 infections. The assay is not intended for use as a screening test for the presence of HSV-1 and HSV-2 in blood or blood products. The assay is for professional use only Simplexa™ HSV 1 & 2 Positive Control Pack The Simplexa™ HSV 1 & 2 Positive Control Pack is intended to be used as a control with the Simplexa™ HSV 1 & 2 Direct kit. This control is not intended for use with other assays or systems.
Sample Types	Cutaneous or mucocutaneous lesion samples	Same
Extraction Methods	None	Same
Assay Methodology	PCR-based system for detecting the presence or absence of viral DNA in clinical specimens.	Same
Detection Techniques	Multiplex assay using different reporter dyes for each target.	Same

Table of Differences		
Name	**Predicate**	**Candidate**
	Lyra® Direct HSV 1 + 2/VZV Assay K133448	**Simplexa™ HSV 1 & 2 Direct**
Assay Targets	HSV-1: glycoprotein G, HSV-2: glycoprotein G, VZV: ORF6: DNA-helicase primase.	Well conserved region of the polymerase genes for HSV-1 and HSV-2.

ANALYTICAL STUDIES
Results of the analytical studies performed were combined with analytical results from K150962.

REPRODUCIBILITY
Reproducibility studies were not conducted again for the purposes of this submission and the results from original submission K150962 are shown below.

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 the positive control and a panel of five contrived sample pools including a low (approximately 1-2 times LoD) and medium positive (approximately 2-4 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 5 different days. Each site had 2 operators; each operator assayed the entire sample panel and positive control once per day, for a total of 2 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)	36.0	2.2	100.0% (30/30)	36.1	2.6	100.0% (30/30)	36.3	2.7	100.0% (90/90)	95.9 to 100.0%
	HSV-1 Medium Positive	100.0% (30/30)	34.4	1.7	100.0% (30/30)	34.8	1.2	100.0% (30/30)	34.6	1.9	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	96.7% (29/30) [a]	NA	NA	98.9% (89/90) [a]	94.0 to 99.8%
	HSV-2 Medium Positive	100.0% (30/30) [a]	NA	NA	96.7% (29/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	98.9% (89/90) [a]	94.0 to 99.8%
	High Negative	96.7% (29/30) [a]	38.8	0.0	93.3% (28/30) [a]	38.7	0.5	90.0% (27/30) [a]	38.0	4.1	93.3% (84/90) [a]	86.2 to 96.9%
	Positive Control	100.0% (30/30)	29.9	0.8	100.0% (30/30)	30.4	1.3	100.0% (29/29)	29.9	2.8	100.0% (89/89)	95.9 to 100.0%
	Total Agreement	**99.4% (179/180)**			**98.3% (177/180)**			**97.8% (175/179)**			**98.5% (531/539)**	**97.1 to 99.2%**

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	96.7% (29/30)[b]	41.1	0.0	98.9% (89/90)[b]	94.0 to 99.8%
	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	100.0% (30/30)	37.4	2.9	90.0% (27/30)	37.5	3.5	93.3% (28/30)	37.1	2.8	94.4% (85/90)	87.6 to 97.6%
	HSV-2 Medium Positive	100.0% (30/30)	35.5	1.9	100.0% (30/30)	35.6	2.0	100.0% (30/30)	35.3	1.6	100.0% (90/90)	95.9 to 100.0%
	High Negative	96.7% (29/30)[b]	39.5	0.0	86.7% (26/30)[b]	38.6	2.9	100.0% (30/30)[b]	NA	NA	94.4% (85/90)[b]	87.6 to 97.6%
	Positive Control	100.0% (30/30)	30.2	1.3	100.0% (30/30)	30.1	0.6	100.0% (29/29)	29.9	1.2	100.0% (89/89)	95.9 to 100.0%
	Total Agreement	**99.4% (179/180)**			**96.1% (173/180)**			**98.9% (176/179)**			**98.0% (528/539)**	**96.4 to 98.9%**

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)	29.8	1.2	100.0% (30/30)	29.7	1.0	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)	29.8	1.4	100.0% (30/30)	29.7	0.9	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Low Positive	100.0% (30/30)	29.6	0.8	100.0% (30/30)	29.8	1.2	100.0% (30/30)	29.7	1.0	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Medium Positive	100.0% (30/30)	29.5	0.6	100.0% (30/30)	29.7	1.4	100.0% (30/30)	29.8	1.4	100.0% (90/90)	95.9 to 100.0%
	High Negative	100.0% (30/30)	29.6	0.6	100.0% (30/30)	29.8	1.2	100.0% (30/30)	29.7	1.0	100.0% (90/90)	95.9 to 100.0%
	Positive Control	100.0% (30/30)	29.5	0.5	100.0% (30/30)	29.7	1.4	100.0% (29/29)	29.7	0.9	100.0% (89/89)	95.9 to 100.0%
	Total Agreement	**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (179/179)**			**100.0% (539/539)**	**96.4 to 98.9%**

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

Analytical Sensitivity/Limit of Detection studies were not conducted again for the purposes of this submission and the results from original submission K150962 are shown below.

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 cutaneous and mucocutaneous swab 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	4	32/32	36.4 ± 1.16
HSV-1 HF	160	32/32	35.2 ± 1.03
HSV-2 G	2	32/32	37.5 ± 1.08
HSV-2 MS	10	31/32	37.9 ± 1.15

ANALYTICAL REACTIVITY / CROSS REACTIVITY

Analytical Reactivity Cutaneous and Mucocutaneous Swab Sample Type

Analytical Reactivity studies were not conducted again for the purposes of this submission and the results from original submission K150962 are shown below.

The analytical reactivity of the Simplexa™ HSV 1 & 2 Direct assay was evaluated using different strains of HSV-1 and HSV-2 that were not used in the determination of the limit of detection (LoD) for the assay. Quantified viral material was spiked into negative cutaneous and mucocutaneous swab matrix containing male and female cutaneous and mucocutaneous swabs using a single dilution and assayed in triplicate. The Simplexa™ HSV 1 & 2 Direct assay was able to detect other strains of HSV-1 and HSV-2 viruses.

HSV Strain/Isolate	Spiked Concentration [$TCID_{50}$/mL]	Qualitative Result (#Detected/#Total)	
		HSV-1	HSV-2
HSV-1 KOS	16	3/3	0/3
HSV-1 F	32	3/3	0/3
HSV-2 Isolate 1	8	0/3	3/3
HSV-2 Isolate 2	8	0/3	3/3
HSV-2 Isolate 3	8	0/3	3/3

Cross Reactivity (Analytical Specificity) Cutaneous and Mucocutaneous Swab Sample Type

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 on cutaneous or mucocutaneous swabs. A total of 71 potential cross-reactants were spiked into negative cutaneous and mucocutaneous swab matrix and assayed in triplicate. No cross-reactivity was observed.

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	Baseline	N/A	35/35	35/35
2	*Acinetobacter calcoaceticus*	1.00 X 10^6 CFU/mL	0/3	0/3
3	*Acinetobacter lwoffii*	1.00 X 10^6 CFU/mL	0/3	0/3
4	*Bacteroides fragilis*	1.00 X 10^6 CFU/mL	0/3	0/3
5	*Bacteroides ureolyticus***	N/A	N/A	N/A
6	*Bordetella bronchiseptica*	1.00 X 10^6 CFU/mL	0/3	0/3
7	*Bordetella pertussis*	1.00 X 10^6 CFU/mL	0/3	0/3
8	*Candida albicans*	1.00 X 10^6 CFU/mL	0/3	0/3
9	*Candida glabrata*	1.00 X 106 CFU/mL	0/3	0/3
10	*Candida guilliermondii*	1.00 x 10^6 CFU/mL	0/3	0/3
11	*Candida krusei*	1.00 x 10^6 CFU/mL	0/3	0/3
12	*Candida lusitaniae*	1.00 x 10^6 CFU/mL	0/3	0/3
13	*Candida parapsilosis*	1.00 x 10^6 CFU/mL	0/3	0/3
14	*Candida tropicalis*	1.00 x 10^6 CFU/mL	0/3	0/3
15	*Chlamydophila pneumoniae*	1.00 x 10^6 IFU/mL	0/3	0/3
16	*Chlamydia trachomatis*	1.00 X 10^6 IFU/mL	0/3	0/3
17	*Clostridium sordellii*	1.00 X 10^6 CFU/mL	0/3	0/3
18	*Clostridium perfringens*	1.00 x 10^6 CFU/mL	0/3	0/3
19	*Corynebacterium genitalium*	1.00 X 10^6 CFU/mL	0/3	0/3
20	Coronavirus (HCoV OC43)	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3
21	*Corynebacterium diphtheriae*	1.00 x 10^6 CFU/mL	0/3	0/3
22	Coxsackievirus B (CVB-1)	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3
23	Cytomegalovirus	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
24	*Enterobacter cloacae*	1.00 x 10^6 CFU/mL	0/3	0/3
25	*Enterococcus faecium*	1.00 x 10^6 CFU/mL	0/3	0/3
26	*Enterococcus faecalis* vanB	1.00 X 10^6 CFU/mL	0/3	0/3
27	Enterovirus 70	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3
28	Enterovirus 71	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
29	Epstein Barr Virus (B95-8)	1.00 X 10^5 copies/mL	0/3	0/3
30	*Escherichia coli* O157H7	1.00 X 10^6 CFU/mL	0/3	0/3
31	*Fusobacterium nucleatum*	1.00 x 10^6 CFU/mL	0/3	0/3
32	*Gardnerella vaginalis*	1.00 X 10^6 CFU/mL	0/3	0/3
33	*Haemophilus ducreyi***	N/A	N/A	N/A
34	*Haemophilus influenzae* (Type A)	1.00 x 10^6 CFU/mL	0/3	0/3
35	Hepatitis B	1.00 X 10^5 IU/mL	0/3	0/3
36	Hepatitis C	1.00 X 10^5 IU/mL	0/3	0/3
37	HHV-6 (Z29 Strain)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
38	HHV-7 SB	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
39	HIV-1 IIIB	1.00 X 10^5 copies/mL	0/3	0/3
40	HIV-2 NIHZ*	Not Available	0/3	0/3
41	HPV18 Recombinant	1.00 X 10^5 PFU/mL	0/3	0/3
42	Human metapneumovirus	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3
43	*Lactobacillus acidophilus*	1.00 X 10^6 CFU/mL	0/3	0/3
44	*Legionella pneumophila*	1.00 x 10^6 CFU/mL	0/3	0/3
45	*Mobiluncus mulieris*	1.00 X 10^6 CFU/mL	0/3	0/3
46	*Moraxella catarrhalis*	1.00 x 10^6 CFU/mL	0/3	0/3
47	*Mycoplasma genitalium***	N/A	N/A	N/A
48	*Mycoplasma hominis*	1.00 X 10^6 CCU/mL	0/3	0/3
49	*Mycoplasma orale***	N/A	N/A	N/A
50	*Mycoplasma pneumoniae*	1.00 x 10^6 CCU/mL	0/3	0/3
51	*Mycoplasma salivarium***	N/A	N/A	N/A
52	*Neisseria gonorrhoeae*	1.00 X 10^6 CFU/mL	0/3	0/3
53	*Neisseria meningitides*	1.00 x 10^6 CFU/mL	0/3	0/3
54	*Prevotella melaninogenica*	1.00 x 10^6 CFU/mL	0/3	0/3
55	*Proteus vulgaris*	1.00 X 10^6 CFU/mL	0/3	0/3
56	Respiratory syncytial virus A	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3
57	Respiratory syncytial virus B	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3
58	Rubella	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
59	*Salmonella enteritidis***	N/A	N/A	N/A
60	*Salmonella typhimurium*	1.00 x 10^6 CFU/mL	0/3	0/3
61	*Staphylococcus aureus* (MRSA), ATCC 700699	1.00 X 10^6 CFU/mL	0/3	0/3
62	*Staphylococcus epidermidis* (MRSE), ATCC 29887	1.00 X 10^6 CFU/mL	0/3	0/3
63	*Streptococcus mutans*	1.00 x 10^6 CFU/mL	0/3	0/3
64	*Streptococcus salivarius*	1.00 x 10^6 CFU/mL	0/3	0/3
65	*Staphylococcus saprophyticus*	1.00 X 10^6 CFU/mL	0/3	0/3
66	*Streptococcus mitis*	1.00 X 10^6 CFU/mL	0/3	0/3
67	*Streptococcus pyogenes*, M1	1.00 X 10^6 CFU/mL	0/3	0/3
68	*Toxoplasma gondii*	1.00 X 10^6 tachyzooites/mL	0/3	0/3
69	*Treponema pallidum***	N/A	N/A	N/A
70	*Trichomonas vaginalis*	1.00 X 10^6 trophozoites/ml	0/3	0/3
71	*Ureaplasma urealyticum*	1.00 X 10^6 CCU/mL	0/3	0/3
72	VZV	1.00 X 10^5 copies/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; 1:50 dilution factor.

** Microorganism was not available for testing therefore *in silico* NCBI BLAST analysis was performed and found no cross reactivity.

N/A = Not applicable

INTERFERENCE

Cutaneous and Mucocutaneous Swab Sample Type

The performance of the Simplexa™ HSV 1 & 2 Direct assay was evaluated with potentially interfering substances that may be present on cutaneous and mucocutaneous swabs at the concentrations indicated in the table below. A total of 24 potentially interfering substances were tested in a low positive HSV-1 and HSV-2 sample (4 times LoD) in negative cutaneous and mucocutaneous swab matrix containing male and female cutaneous and mucocutaneous swabs and assayed in triplicate. No interference was observed.

Potential Interferent	Interferent Concentration	# Detected/# Total	
		HSV-1	HSV-2
Acetaminophen	7% w/v	3/3	3/3
Albumin	10 mg/mL	3/3	3/3
Buffy coat	7% v/v	3/3	3/3
Carmex® Original Lip Balm (Camphor, 1.7%; Menthol, 0.7%)	10% v/v	3/3	3/3
Casein	10 mg/mL	3/3	3/3
Chlorpheniramine maleate	5 mg/mL	3/3	3/3
Cold-EEZE® Cold Remedy plus Throat (Zincum Gluconicum 2X)	10% v/v	3/3	3/3
Cornstarch	1.25 mg/mL	3/3	3/3
Desitin (Zinc Oxide, 40%)	7% w/v	3/3	3/3
Dextromethorphan hydrobromide	10 mg/mL	3/3	3/3
Foscarnet	1.25 mg/mL	3/3	3/3
Ganciclovir	2.5 mg/mL	3/3	3/3
Lanacane Benzethonium chloride, 0.2%; Benzocaine, 20%)	7% v/v	3/3	3/3
Lip Clear® Lysine (Zinc Oxide, 1.2%)	7% w/v	3/3	3/3
Listerine (Eucalyptol, 0.092%; Menthol, 0.042%; Methyl salicylate, 0.060%; Thymol, 0.064%)	7% v/v	3/3	3/3
Miconazole 3 (Miconazole nitrate, 2%)	7% w/v	3/3	3/3
Seminal fluid	7% w/v	3/3	3/3
Spermicide	7% w/v	3/3	3/3
Tioconazole	7% w/v	3/3	3/3
Toothpaste	7% w/v	3/3	3/3
Valganciclovir	2.5 mg/mL	3/3	3/3
Whole Blood	10% v/v	3/3	3/3
Urine	10% v/v	3/3	3/3
KY Jelly	5% v/v	3/3	3/3

COMPETITIVE INTERFERENCE Cutaneous and Mucocutaneous Swab Sample Type
Competitive interference studies were not conducted again for the purposes of this submission and the results from original submission K150962 are shown below.

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. Baseline sample results are also shown below. No competitive interference was observed.

Baseline (Low Level)		Competitive Interferent (High Concentration)		Qualitative Results (#Detected/#Total)	
Strain	Concentration ($TCID_{50}$/mL)	Strain	Concentration ($TCID_{50}$/mL)	HSV-1	HSV-2
HSV-1 McIntyre	16	HSV-2 G	0	5/5	0/5
HSV-1 McIntyre	16	HSV-2 G	1.00×10^6	3/3	3/3
HSV-2 G	8	HSV-1 McIntyre	0	0/5	5/5
HSV-2 G	8	HSV-1 McIntyre	1.71×10^3	3/3	3/3

INHIBITION BY OTHER MICROORGANISMS – Cutaneous and Mucocutaneous Swab Sample Type
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 71 potentially inhibitory organisms was individually spiked into a pool with a low concentration (approximately 4 times LoD) of HSV-1 and HSV-2 in cutaneous and mucocutaneous swab matrix. 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 would be 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". *In silico* NCBI BLAST analysis was performed for 7 potential inhibitory organisms.

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	Baseline	Not Applicable	35/35	35/35
2	*Acinetobacter calcoaceticus*	1.00×10^6 CFU/mL	3/3	3/3
3	*Acinetobacter lwoffii*	1.00×10^6 CFU/mL	3/3	3/3
4	*Bacteroides fragilis*	1.00×10^6 CFU/mL	3/3	3/3
5	*Bacteroides ureolyticus***	N/A	N/A	N/A
6	*Bordetella bronchiseptica*	1.00×10^6 CFU/mL	3/3	3/3
7	*Bordetella pertussis*	1.00×10^6 CFU/mL	3/3	3/3
8	*Candida albicans*	1.00×10^6 CFU/mL	3/3	3/3
9	*Candida glabrata*	1.00×10^6 CFU/mL	3/3	3/3
10	*Candida guilliermondii*	1.00×10^6 CFU/mL	3/3	3/3
11	*Candida krusei*	1.00×10^6 CFU/mL	3/3	3/3

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
12	*Candida lusitaniae*	1.00 x 10^6 CFU/mL	3/3	3/3
13	*Candida parapsilosis*	1.00 x 10^6 CFU/mL	3/3	3/3
14	*Candida tropicalis*	1.00 x 10^6 CFU/mL	3/3	3/3
15	*Chlamydophila pneumoniae*	1.00 x 10^6 IFU/mL	3/3	3/3
16	*Chlamydia trachomatis*	1.00 X 10^6 IFU/mL	3/3	3/3
17	*Clostridium sordellii*	1.00 X 10^6 CFU/mL	3/3	3/3
18	*Clostridium perfringens*	1.00 x 10^6 CFU/mL	3/3	3/3
19	*Corynebacterium genitalium*	1.00 X 10^6 CFU/mL	3/3	3/3
20	Coronavirus (HCoV OC43)	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3
21	*Corynebacterium diphtheriae*	1.00 x 10^6 CFU/mL	3/3	3/3
22	Coxsackievirus B (CVB-1)	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3
23	Cytomegalovirus	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
24	*Enterobacter cloacae*	1.00 x 10^6 CFU/mL	3/3	3/3
25	*Enterococcus faecium*	1.00 x 10^6 CFU/mL	3/3	3/3
26	*Enterococcus faecalis* vanB	1.00 X 10^6 CFU/mL	3/3	3/3
27	Enterovirus 70	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3
28	Enterovirus 71	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
29	Epstein Barr Virus (B95-8)	1.00 X 10^5 copies/mL	3/3	3/3
30	*Escherichia coli* O157H7	1.00 X 10^6 CFU/mL	3/3	3/3
31	*Fusobacterium nucleatum*	1.00 x 10^6 CFU/mL	3/3	3/3
32	*Gardnerella vaginalis*	1.00 X 10^6 CFU/mL	3/3	3/3
33	*Haemophilus ducreyi***	N/A	N/A	N/A
34	*Haemophilus influenzae* (Type A)	1.00 x 10^6 CFU/mL	3/3	3/3
35	Hepatitis B	1.00 X 10^5 IU/mL	3/3	3/3
36	Hepatitis C	1.00 X 10^5 IU/mL	3/3	3/3
37	HHV-6 (Z29 Strain)	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
38	HHV-7 SB	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
39	HIV-1 IIIB	1.00 X 10^5 copies/mL	3/3	3/3
40	HIV-2 NIHZ*	N/A	3/3	3/3
41	HPV18 Recombinant	1.00 X 10^5 PFU/mL	3/3	3/3
42	Human metapneumovirus	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3
43	*Lactobacillus acidophilus*	1.00 X 10^6 CFU/mL	3/3	3/3
44	*Legionella pneumophila*	1.00 x 10^6 CFU/mL	3/3	3/3
45	*Mobiluncus mulieris*	1.00 X 10^6 CFU/mL	3/3	3/3
46	*Moraxella catarrhalis*	1.00 x 10^6 CFU/mL	3/3	3/3
47	*Mycoplasma genitalium***	N/A	N/A	N/A
48	*Mycoplasma hominis*	1.00 X 10^6 CCU/mL	3/3	3/3
49	*Mycoplasma orale***	Not Applicable	N/A	N/A
50	*Mycoplasma pneumoniae*	1.00 x 10^6 CCU/mL	4/8	5/8

No.	Microorganism	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
51	*Mycoplasma salivarium***	N/A	N/A	N/A
52	*Neisseria gonorrhoeae*	1.00 X 10^6 CFU/mL	3/3	3/3
53	*Neisseria meningitides*	1.00 x 10^6 CFU/mL	3/3	3/3
54	*Prevotella melaninogenica*	1.00 x 10^6 CFU/mL	3/3	3/3
55	*Proteus vulgaris*	1.00 X 10^6 CFU/mL	3/3	3/3
56	Respiratory syncytial virus A	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3
57	Respiratory syncytial virus B	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3
58	Rubella	1.00 X 10^5 $TCID_{50}$/mL	3/3	3/3
59	*Salmonella enteritidis***	N/A	N/A	N/A
60	*Salmonella typhimurium*	1.00 x 10^6 CFU/mL	3/3	3/3
61	*Staphylococcus aureus* (MRSA), ATCC 700699	1.00 X 10^6 CFU/mL	3/3	3/3
62	*Staphylococcus epidermidis* (MRSE), ATCC 29887	1.00 X 10^6 CFU/mL	3/3	3/3
63	*Streptococcus mutans*	1.00 x 10^6 CFU/mL	3/3	3/3
64	*Streptococcus salivarius*	1.00 x 10^6 CFU/mL	3/3	3/3
65	*Staphylococcus saprophyticus*	1.00 X 10^6 CFU/mL	3/3	3/3
66	*Streptococcus mitis*	1.00 X 10^6 CFU/mL	3/3	3/3
67	*Streptococcus pyogenes*, M1	1.00 X 10^6 CFU/mL	3/3	3/3
68	*Toxoplasma gondii*	1.00 X 10^6 tachyzooites/mL	3/3	3/3
69	*Treponema pallidum***	N/A	N/A	N/A
70	*Trichomonas vaginalis*	1.00 X 10^6 trophozoites/ml	3/3	3/3
71	*Ureaplasma urealyticum*	1.00 X 10^6 CFU/mL	3/3	3/3
72	VZV	1.00 X 10^5 copies/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; 1:50 dilution factor.

**Microorganism was not available for testing therefore *in silico* NCBI BLAST analysis was performed and found no inhibition.

N/A = Not applicable.

CLINICAL AGREEMENT

Prospective Study 1 – Cutaneous and Mucocutaneous Swab Sample Type K150962
A total of 718 cutaneous and mucocutaneous lesion swab samples were prospectively collected May 28, 2014 through December 4, 2014 from patients with signs and symptoms of herpes simplex virus (HSV) infection from 6 geographically diverse locations. Of the 718 samples collected, 9 samples were removed from the analysis because they were either not tested or had invalid results on the 3 assays (Simplexa™ HSV 1 & 2 Direct, culture, or bi-directional sequencing). Of the 709 remaining samples, 13 samples were removed from the analysis because they were not tested on the tests included in the composite comparator method sufficient to generate a final comparator result. A total of 696 samples were used for the analysis. Samples were tested on Simplexa™ HSV 1 & 2 Direct at the collection sites, culture samples were sent to a central lab, and the sequencing samples were sent to DiaSorin Molecular. All samples were either tested fresh or frozen within 72 hours of sample collection. Aliquots were made from each sample; 1 aliquot was tested on Simplexa™ HSV 1 & 2 Direct at the collection site, and the remainder was sent to DiaSorin Molecular. One aliquot was sent for culture testing at an external site, 1 aliquot was used for bi-directional sequencing, and the last aliquot was held as a retain. Any sample that was not collected and frozen within 72 hours was disqualified.

The testing included 332 total runs with 638 evaluable controls, and 5 invalid control pairs. There were 718 patient samples of which 2 were not evaluable due to internal control failure, 5 not evaluable due to invalid runs, 1 was not evaluable due to wrong sample type, 1 not evaluable due to testing on a commercial instrument, 5 Insufficient Volume Errors, 3 not evaluable because of daily control issues, and 5 non-enrollable patient samples not meeting acceptance criteria (tested >72hrs post collection).

Prospective Study 2 – Cutaneous and Mucocutaneous Swab Sample Type K173798
A total of 514 cutaneous and mucocutaneous lesion swab samples were prospectively collected July 24, 2017 through October 11, 2017. These samples were collected from patients with signs and symptoms of herpes simplex virus (HSV) infection from 4 geographically diverse sites. Of the 514 samples, 511 samples were evaluable on Simplexa™ HSV 1 & 2 Direct, 512 were evaluable by the culture method, and 510 were evaluable by the bi-directional sequencing method. Samples were tested on Simplexa™ HSV 1 & 2 Direct at the collection sites, culture samples were sent to a central lab, and the sequencing samples were sent to DiaSorin Molecular. All samples were either tested fresh or frozen within 72 hours of sample collection. Aliquots were made from each sample; 1 aliquot was tested on Simplexa™ HSV 1 & 2 Direct at the collection site, and the remainder was sent to DiaSorin Molecular. One aliquot was sent for culture testing at an external site, 1 aliquot was used for bi-directional sequencing, and the last aliquot was held as a retain. Any sample that was not collected and frozen within 72 hours was disqualified.

The testing included 153 total Runs with 250 evaluable controls, and 3 invalid control pairs. There were 514 patient samples of which 2 were not evaluable due to EC505 codes from both the HSV-1 (FAM) & HSV-2 (CFR610) channel, 2 Insufficient Volume errors, 18 samples not evaluable because of daily control issues (7 samples were non-evaluable due to invalid PC control runs and 11 samples were non-evaluable due to no daily control runs) and 1 was a non-enrollable patient sample that did not meet the acceptance criteria (tested >72hrs post collection).

Retrospective Study – Cutaneous and Mucocutaneous Swab Sample Type
A total of 174 Cutaneous HSV-1 swabs, 174 Mucocutaneous HSV-2 swabs and 17 samples from unknown locations, were retrospectively collected June 6, 2011 to May 17, 2014 and February 21, 2017 through July 17, 2017. All samples were tested using the composite comparator method.

The clinical performance of the Simplexa HSV 1 & 2 Direct assay was evaluated by comparing the positive and negative percent agreement to a composite comparator algorithm consisting of culture, bi-directional sequencing and a FDA cleared NAAT. All samples yielding a positive result by either sequencing or culture were tested on an FDA cleared NAAT and a 2 out of 3 rule was used to determine the final composite results. All sites collected and tested the swab samples on the Simplexa™ HSV-1 and HSV-2 Direct and sent samples to a central lab for culture testing. For culture, each sample was

tested for HSV- 2 first and if positive for HSV-2 no further testing was performed. Samples that were HSV-2 culture negative were further tested for HSV-1 culture positivity. Dual positives could not be identified in the culture assay.

The available retained samples were sent to DiaSorin Molecular and tested in a validated bi-directional sequencing assay. Results for Simplexa™ HSV 1 & 2 Direct compared to the composite comparator algorithm are presented in the tables that follow when combined with the data from the second sample set.

Prospective Results Composite Reference Method HSV-1 Cutaneous Swabs
The table below shows HSV-1 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method based on the observed prevalence in the study population for HSV-1 for cutaneous swabs.

Clinical Agreement - (Cutaneous for HSV-1)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	30	7	37
Not Detected	0	182	182
Total	30	189	219
%PPA	100.0%(30/30) 95% CI: 88.7% to 100.0%	%NPA	96.3%(182/189) 95% CI: 92.6% to 98.5%

Prospective Results Composite Reference Method HSV-1 Mucocutaneous Swabs
The table below shows HSV-1 positive and negative percent Agreement (PPA and NPA) vs. Composite Reference Method based on the observed prevalence in the study population for HSV-1 for Mucocutaneous swabs.

Clinical Agreement - (Mucocutaneous for HSV-1)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	162	18	180
Not Detected	3	703	706
Total	165	721	887
%PPA	98.2%(162/165) 95% CI: 94.4% to 99.6%	%NPA	97.5%(703/721) 95% CI: 96.1% to 98.4%

Prospective Results Composite Reference Method HSV-2 Cutaneous Swabs

The table below shows HSV-2 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method based on the observed prevalence in the study population for HSV-2 for cutaneous swabs.

Clinical Agreement - (Cutaneous for HSV-2)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	32	4	36
Not Detected	1	182	183
Total	33	186	219
%PPA	97.0%(32/33) 95% CI: 84.4% to 99.5%	%NPA	97.9%(182/186) 95% CI: 94.6% to 99.2%

Prospective Results Composite Reference Method HSV-2 Mucocutaneous Swabs

The table below shows HSV-2 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method based on the observed prevalence in the study population for HSV-2 for mucocutaneous swabs.

Clinical Agreement - (Mucocutaneous for HSV-2)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	193	23	216
Not Detected	1	669	670
Total	194	692	886
%PPA	99.5%(193/194) 95% CI: 97.1% to 100.0%	%NPA	96.7%(669/692) 95% CI: 95.1% to 97.8%

Retrospective Results Composite Reference Method HSV-1 Cutaneous Swabs

The table below shows HSV-1 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method for retrospectively collected HSV-1 for cutaneous swabs.

Clinical Agreement - (Cutaneous for HSV-1)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	26	1	27
Not Detected	0	91	91
Total	26	92	118
%PPA	100.0%(26/26) 95% CI: 87.1% to 100.0%	%NPA	98.9%(91/92) 95% CI: 94.1% to 99.8%

Retrospective Results Composite Reference Method HSV-1 Mucocutaneous Swabs

The table below shows HSV-1 positive and negative percent Agreement (PPA and NPA) vs. Composite Reference Method for retrospectively collected HSV-1 for mucocutaneous swabs.

Clinical Agreement - (Mucocutaneous for HSV-1)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	32	1	33
Not Detected	0	113	113
Total	32	114	146
%PPA	100.0%(32/32) 95% CI: 89.3% to 100.0%	%NPA	99.1%(113/114) 95% CI: 95.2% to 100.0%

Retrospective Results Composite Reference Method HSV-2 Cutaneous Swabs
The table below shows HSV-2 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method for retrospectively collected HSV-2 for cutaneous swabs.

Clinical Agreement - (Cutaneous for HSV-2)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	29	0	29
Not Detected	0	89	89
Total	29	89	118
%PPA	100.0%(29/29) 95% CI: 88.3% to 100.0%	%NPA	100.0%(89/89) 95% CI: 95.9% to 100.0%

Retrospective Results Composite Reference Method HSV-2 Mucocutaneous Swabs
The table below shows HSV-2 positive and negative percent Agreement (PPA and NPA) vs. Composite Reference Method for retrospectively collected HSV-2 for mucocutaneous swabs.

Clinical Agreement - (Mucocutaneous for HSV-2)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	22	0	22
Not Detected	0	124	124
Total	22	124	146
%PPA	100.0%(22/22) 95% CI: 85.1% to 100.0%	%NPA	100.0%(124/124) 95% CI: 97.0% to 100.0%

Retrospective Results Composite Reference Method HSV-1 Unknown Locations Cutaneous Swabs
The table below shows HSV-1 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method for retrospectively collected HSV-1 for cutaneous swabs from unknown locations.

Clinical Agreement - (Unknown Sample Locations for HSV-1)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	3	0	3
Not Detected	0	12	12
Total	3	12	15
%PPA	100.0%(3/3) 95% CI: 43.9% to 100.0%	%NPA	100.0%(12/12) 95% CI: 75.8% to 100.0%

Retrospective Results Composite Reference Method HSV-2 Unknown Locations Cutaneous Swabs
The table below shows HSV-2 positive and negative percent agreement (PPA and NPA) vs. Composite Reference Method for retrospectively collected HSV-2 for cutaneous swabs from unknown locations.

Clinical Agreement - (Unknown Sample Locations for HSV-2)			
Simplexa™ HSV 1 & 2 Direct Results	**Composite Reference Method**		
	Detected	**Not Detected**	**Total**
Detected	3	0	3
Not Detected	0	12	12
Total	3	12	15
%PPA	100.0%(3/3) 95% CI: 43.9% to 100.0%	%NPA	100.0%(12/12) 95% CI: 75.8% to 100.0%

EXPECTED VALUES – Cutaneous and Mucocutaneous Swabs

The observed expected values using the Simplexa™ HSV 1 & 2 Direct assay are presented below. The data is stratified by age, gender and lesion location.

Cutaneous and Mucocutaneous Lesion Swabs by Age and Gender

Gender	Age Group	Total	Simplexa™ HSV 1& 2 Direct HSV-1 Results		Simplexa™ HSV 1& 2 Direct HSV-2 Results	
			Positive	Prevalence	Positive	Prevalence
Female	≤18 years	128	24	18.8%	20	15.6%
	>18 to 21 years	96	34	35.4%	24	25.0%
	>21 years	779	141	18.1%	204	26.2%
	All	1003	199	19.8%	248	24.7%
Male	≤18 years	36	11	30.6%	1	2.8%
	>18 to 21 years	31	14	45.2%	7	22.6%
	>21 years	142	19	13.4%	28	19.7%
	All	209	44	21.1%	36	17.2%
All		1212	243	20.0%	284	23.4%

Cutaneous and Mucocutaneous Lesion Swabs by Lesion Location

Skin Type	Lesion Location	Total Specimens	Simplexa™ HSV 1& 2 Direct HSV-1 Results		Simplexa™ HSV 1& 2 Direct HSV-2 Results	
			Positive	Prevalence	Positive	Prevalence
Cutaneous	Genital	136	28	20.6%	31	22.8%
	Skin	92	12	13.0%	7	7.6%
	All	228	40	17.5%	38	16.7%
Mucocutaneous	Anorectal	16	6	37.5%	2	12.5%
	Genital	854	167	19.6%	232	27.2%
	Nasal	3	1	33.3%	0	0.0%
	Ocular	11	2	18.2%	0	0.0%
	Oral	51	20	39.2%	1	2.0%
	Unknown	2	0	0.0%	0	0.0%
	Urethra	3	0	0.0%	0	0.0%
	All	940	196	20.9%	235	25.0%
Unknown	Unknown	44	7	15.9%	11	25.0%
	All	44	7	15.9%	11	25.0%
All		1212	243	20.0%	284	23.4%

CARRY-OVER CONTAMINATION

The amplification carry-over for the Simplexa™ assays including the Simplexa™ HSV 1 & 2 Direct assay was assessed from the Simplexa™ Flu A/B & RSV Direct REF MOL2650 (K120413) viral 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 adjacent to strong positive samples. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was observed.

FRESH VS FROZEN

Fresh versus frozen studies were not performed for the purposed on the subject 510K submission and data from the original submission K150962 was used (see below).

Storage conditions were validated using the following transport media types BD VTM, M4, M4RT, M5, M6, and UTM by spiking media with organism at concentrations ranging from 3 times LoD to 50 times LoD and at different storage temperatures and durations. The validated storage for samples was found to be the following;

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.