DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

FOCUS DIAGNOSTICS, INC.
SHARON YOUNG
SENIOR REGULATORY AFFAIRS SPECIALIST
11331 VALLEY VIEW STREET
CYPRESS, CA 90630

August 28, 2015

Re: K150962
Trade/Device Name: Simplexa HSV 1 & 2 Direct, Simplexa HSV1 & 2 Positive Control Pack
Regulation Number: 21 CFR 866.3305
Regulation Name: Herpes simplex virus serological assays
Regulatory Class: II
Product Code: OQO
Dated: August 25, 2015
Received: August 26, 2015

Dear Ms. 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 Parts 801 and 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.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. 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.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Stephen J. Lovell -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: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K150962

Device Name

Simplexa™ HSV 1 & 2 Direct and Simplexa™ HSV 1 & 2 Positive Control Pack

Indications for Use *(Describe)*

Simplexa™ HSV 1 & 2 Direct
The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct assay is intended for use on the 3M Integrated Cycler instrument for the qualitative detection and differentiation of herpes simples virus (HSV-1 and HSV-2) DNA present in genital lesion swabs samples from patients with signs and symptoms of HSV-1 or HSV-2 infection of the genitalia. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 genital 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.

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.

Applicant	Focus Diagnostics, Inc. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Sharon Young tel 562.240.6680 fax 562.240.6529 syoung@focusdx.com
Summary Date	August 20, 2015
Proprietary Name	Simplexa™ HSV 1 & 2 Direct Simplexa™ HSV 1 & 2 Positive Control Pack
Generic Name	Herpes Simplex Virus serological assays
Classification **Regulation** **Product Code**	OQO
Predicate Device	OQO - *artus*® HSV-1/2 QS-RGQ MDx Kit (K142738)

Intended Use

Simplexa™ HSV 1 & 2 Direct

The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct assay is intended for use on the 3M Integrated Cycler instrument for the qualitative detection and differentiation of herpes simples virus (HSV-1 and HSV-2) DNA present in genital lesion swabs samples from patients with signs and symptoms of HSV-1 or HSV-2 infection of the genitalia. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 genital 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 genital swab specimens without nucleic acid extraction. The system consists of the Simplexa™ HSV 1 & 2 Direct assay, the 3M Integrated Cycler (with 3M Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories.

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

Predicate Device Information

Similarities

Name	Simplexa™ HSV 1 & 2 Direct	*artus*® HSV-1/2 QS-RGQ MDx Kit K142738
Intended Use	Simplexa™ HSV 1 & 2 Direct The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct assay is intended for use on the 3M Integrated Cycler instrument for the qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA present in genital lesion swabs samples from patients with signs and symptoms of HSV-1 or HSV-2 infection of the genitalia. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 genital 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.	The artus HSV-1/2 QS-RGQ MDx Kit is an in vitro real-time PCR DNA amplification assay performed on the QIAsymphony RGQ MDx system for the direct qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA in genital or oral vesicular lesions from male and female patients suspected of HSV infection. The assay is intended for use as an aid in diagnosis of HSV infection in symptomatic patients. Warning: The artus HSV-1/2 QS-RGQ MDx Kit is not FDA-cleared for use with cerebrospinal fluid (CSF) or for prenatal screening.
Assay Targets	HSV-1 and HSV-2 DNA	HSV-1 and HSV-2 DNA
Detection Techniques	Multiplex assay using different reporter dyes for each target.	Multiplex assay using different reporter dyes for each target.

Differences

Name	Simplexa™ HSV 1 & 2 Direct	*artus*® HSV-1/2 QS-RGQ MDx Kit K142738
Sample Types	Genital herpetic lesion swab samples	Male and female genital or oral herpetic lesions.
Assay Methodology	PCR-based system for detecting the presence / absence of viral DNA in clinical specimens	Amplification and detection are carried out using the artus HSV-1/2 QS-RGQ MDx Kit with the Rotor-Gene Q MDx (RGQ MDx) and Rotor-Gene Assay Manager software.

REPRODUCIBILITY – Genital Swab Sample Type

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 five different days. Each site had two operators; each operator assayed the entire sample panel and positive control once per day, for a total of two sets of data per day. Combined results for all sites are presented in the tables below.

	Sample	Site – 1			Site – 2			Site – 3			Total % Agreement with Expected Results	95% CI
		% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV		
HSV-1 Result	HSV-1 Low Positive	100.0% (30/30)	36.0	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 – Genital Swab Sample Type

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 genital swab matrix containing male and female genital swabs. 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 –Genital Swab Sample Type

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 genital swab matrix containing male and female genital 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) – Genital 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 swabs of the genital region. Thirty six (36) potential cross-reactants were spiked into negative genital swab matrix containing male and female genital swabs and assayed in triplicate. No cross-reactivity was observed.

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	None (Baseline)	Not Applicable	0/15	0/15
2	*Bacteroides fragilis*	1.00 X 10^6 cfu/mL	0/3	0/3
3	*Bacteroides ureolyticus**	Not Applicable	Not Applicable	Not Applicable
4	*Candida albicans*	1.00 X 10^6 cfu/mL	0/3	0/3
5	*Chlamydia trachomatis*	1.00 X 10^6 IFU/mL	0/3	0/3
6	*Clostridium sordellii*	1.00 X 10^6 cfu/mL	0/3	0/3
7	*Corynebacterium genitalium*	1.00 X 10^6 cfu/mL	0/3	0/3
8	Cytomegalovirus AD169 strain	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
9	*Enterococcus faecalis* vanB	1.00 X 10^6 cfu/mL	0/3	0/3
10	Enterovirus 71	1.00 X 10^5 $TCID_{50}$/mL	0/8	1/8
11	Epstein Barr Virus (B95-8)	1.00 X 10^5 copies/mL	0/3	0/3
12	*Escherichia coli* O157H7	1.00 X 10^6 cfu/mL	0/3	0/3
13	*Gardnerella vaginalis*	1.00 X 10^6 cfu/mL	0/3	0/3
14	Hepatitis B	1.00 X 10^5 IU/mL	0/3	0/3
15	Hepatitis C	1.00 X 10^5 IU/mL	0/3	0/3
16	HHV-6 (Z29 Strain)	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
17	HHV-7 SB	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
18	HIV-1 IIIB	1.00 X 10^5 copies/mL	0/3	0/3

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
19	HIV-2 NIHZ**	Not Available	0/3	0/3
20	HPV18 Recombinant	1.00 X 10^5 pfu/mL	0/3	0/3
21	*Lactobacillus acidophilus*	1.00 X 10^6 cfu/mL	0/3	0/3
22	*Mobiluncus mulieris*	1.00 X 10^6 cfu/mL	0/3	0/3
23	*Mycoplasma genitalium***	Not Applicable	Not Applicable	Not Applicable
24	*Mycoplasma hominis*	1.00 X 10^6 CCU/mL	0/3	0/3
25	*Neisseria gonorrhoeae*	1.00 X 10^6 cfu/mL	0/3	0/3
26	*Proteus vulgaris*	1.00 X 10^6 cfu/mL	0/3	0/3
27	Rubella	1.00 X 10^5 $TCID_{50}$/mL	0/3	0/3
28	*Staphylococcus aureus* (MRSA), ATCC 700699	1.00 X 10^6 cfu/mL	0/3	0/3
29	*Staphylococcus epidermidis* (MRSE), ATCC 29887	1.00 X 10^6 cfu/mL	0/3	0/3
30	*Staphylococcus saprophyticus*	1.00 X 10^6 cfu/mL	0/3	0/3
31	*Streptococcus mitis*	1.00 X 10^6 cfu/mL	0/3	0/3
32	*Streptococcus pyogenes*, M1	1.00 X 10^6 cfu/mL	0/3	0/3
33	*Toxoplasma gondii*	1.00 X 10^6 tachyzooites/mL	0/3	0/3
34	*Treponema pallidum***	Not Applicable	Not Applicable	Not Applicable
35	*Trichomonas vaginalis*	1.00 X 10^6 trophozoites/ml	0/3	0/3
36	*Ureaplasma urealyticum*	1.00 X 10^6 CCU/mL	0/3	0/3
37	VZV	1.00 X 10^5 copies/mL	0/3	0/3

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

INTERFERENCE – Genital Swab Sample Type

The performance of the Simplexa™ HSV 1 & 2 Direct assay was evaluated with potentially interfering substances that may be present on swabs of the genital region at the concentrations indicated in the table below. A total of five (26) potentially interfering substances were tested in a low positive HSV-1 and HSV-2 sample (4 times LoD) in negative genital swab matrix containing male and female genital swabs and assayed in triplicate. No interference was observed.

Potential Interferent	Interferent Concentration	#Detected/#Total		
		HSV-1	HSV-2	IC
Abreva cold sore treatment	7% w/v	3/3	3/3	3/3
Acyclovir	2.5 mg/mL	3/3	3/3	3/3
Acyclovir Cream*	7% w/v	8/8	5/8	8/8
Albumin	10 mg/mL	3/3	3/3	3/3
Balneol Hygienic Cleansing lotion	7% w/v	3/3	3/3	3/3
Casein	10 mg/mL	3/3	3/3	3/3
Cidofovir	2.5 mg/mL	3/3	3/3	3/3
Clotrimazole vaginal cream	7% w/v	3/3	3/3	3/3
Denavir	2.5 mg/mL	3/3	3/3	3/3

Potential Interferent	Interferent Concentration	#Detected/#Total		
		HSV-1	HSV-2	IC
Douche	7% w/v	3/3	3/3	3/3
Famciclovir	2.5 mg/mL	3/3	3/3	3/3
Feces	2.5 mg/mL	3/3	3/3	3/3
Gynol II (Contraceptive jelly)	7% w/v	3/3	3/3	3/3
KY Jelly	5% v/v	3/3	3/3	3/3
Monistat 1	7% w/v	3/3	3/3	3/3
Monistat 3	7% w/v	3/3	3/3	3/3
Mucin	7% w/v	3/3	3/3	3/3
Preparation H Hemorrhoid cream	7% w/v	3/3	3/3	3/3
Releev cold sore treatment	7% w/v	3/3	3/3	3/3
Urine	10% v/v	3/3	3/3	3/3
Vagicaine Anti-Itch Cream	7% w/v	3/3	3/3	3/3
Vagisil creme	7% w/v	3/3	3/3	3/3
VagiStat 1	7% w/v	3/3	3/3	3/3
Valacyclovir	2.5 mg/mL	3/3	3/3	3/3
Whole Blood	10% v/v	3/3	3/3	3/3
YeastGard Suppositories	7% w/v	3/3	3/3	3/3
* One out of three (1/3) initial replicates and two out of five (2/5) confirmation replicates were negative for HSV-2.				

CLINICAL AGREEMENT – Genital Swab Sample Type

Prospective Study
A total of 718 genital swab samples were prospectively collected from patients with signs and symptoms of genital 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. The clinical performance of the Simplexa HSV 1 & HSV 2 assay was evaluated by comparing the positive and negative percent agreement to a composite comparator algorithm consisting of; culture, bi-directional sequencing and an FDA cleared NAAT. A positive result for HSV-1 and/or HSV-2 was determined by a positive test result in either the culture or the bi-directional sequencing. If both the culture and the bi-directional sequencing yielded positive results but disagreed in the differentiation of HSV-1 versus HSV-2, the results of the FDA cleared NAAT were used and a 2 out of 3 rule was followed to determine the type of the virus (e.g. if two of the methods were positive for HSV-1, the final comparator result was HSV-1 positive). All sites collected and tested the genital 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 Focus Diagnostics and tested in a validated bi-directional sequencing assay. Of the 24 discordant samples that were positive for HSV-2 by the culture method but positive for HSV-1 by the bi-directional sequencing assay, 18 samples had valid results and 1 sample had an invalid result when tested on an FDA cleared NAAT. There were 5 samples that were not tested on the FDA cleared NAAT due to insufficient volume and therefore 6 out of 24 samples were excluded from the

analysis. There were 2 samples that were positive for HSV-1 by the culture method but positive for HSV-2 by the bi-directional sequencing assay that were not tested on the FDA cleared NAAT for insufficient volume and therefore were excluded from analysis. Results for Simplexa™ HSV 1 & 2 Direct compared to the composite comparator algorithm are presented in the following tables.

Simplexa™ HSV 1 & 2 Direct Compared to Composite Comparator Result (HSV-1)

Simplexa™ HSV 1 & 2 Direct Results HSV-1	Composite Comparator Result (HSV-1)		
	Detected	**Not Detected**	**Total**
Detected	111	10	121
Not Detected	3	560	563
Total	114	570	684
Sensitivity	97.4%(111/114) 95% CI: 92.5% to 99.1%	Specificity	98.2%(560/570) 95% CI: 96.8% to 99.0%
PPV	91.7%(111/121) 95% CI: 85.5% to 95.4%	NPV	99.5%(560/563) 95% CI: 98.4% to 99.8%

Simplexa™ HSV 1 & 2 Direct Compared to Composite Comparator Result (HSV-2)

Simplexa™ HSV 1 & 2 Direct Results HSV-2	Composite Comparator Result (HSV-2)		
	Detected	**Not Detected**	**Total**
Detected	175	11	186
Not Detected	5	497	502
Total	180	508	688
Sensitivity	97.2%(175/180) 95% CI: 93.7% to 98.8%	Specificity	97.8%(497/508) 95% CI: 96.2% to 98.8%
PPV	94.1%(175/186) 95% CI: 89.7% to 96.7%	NPV	99.0%(497/502) 95% CI: 97.7% to 99.6%

Retrospective Study
A total of 28 genital swab samples (14 positive HSV-1 and 14 positive HSV-2) were retrospectively collected from male patients with signs and symptoms of genital herpes simplex virus (HSV) infection and contained preselected positive and negative samples. The samples were tested at Focus Diagnostics using the Simplexa™ HSV 1 & 2 Direct and a validated bi-directional sequencing assay.

HSV-1 Validated Bi-Directional Sequencing Assay Results

Simplexa™ HSV 1 & 2 Direct Results	Composite Comparator Result (HSV-1)		
	Detected	Not Detected	Total
Detected	14	1	15
Not Detected	0	13	13
Total	14	14	28
PPA	100.0%(14/14) 95% CI: 78.5% to 100.0%	NPA	92.9%(13/14) 95% CI: 68.5% to 98.7%
PPV	93.3%(14/15) 95% CI: 70.2% to 98.8%	NPV	100.0%(13/13) 95% CI: 77.2% to 100.0%

HSV-2 Validated Bi-Directional Sequencing Assay Results

Simplexa™ HSV 1 & 2 Direct Results	Composite Comparator Result (HSV-2)		
	Detected	Not Detected	Total
Detected	14	0	14
Not Detected	0	14	14
Total	14	14	28
PPA	100.0%(14/14) 95% CI: 78.5% to 100.0%	NPA	100.0%(14/14) 95% CI: 78.5% to 100.0%
PPV	100.0%(14/14) 95% CI: 78.5% to 100.0%	NPV	100.0%(14/14) 95% CI: 78.5% to 100.0%

EXPECTED VALUES – Genital Swab Sample Type

The observed expected values using the Simplexa™ HSV 1 & 2 Direct assay are presented below for patients 17 years of age or older from the prospective study population. The data is stratified by age, and gender and collection sites.

Gender	Age Group	Sample Demographics by Simplexa™ HSV 1 & 2 Direct Assay					
		All	Simplexa™ HSV 1 & 2 Direct Result				
			HSV-1 & HSV-2 Not Detected	HSV-1 Detected & HSV-2 Not Detected	HSV-1 Not Detected & HSV-2 Detected	HSV-1 & HSV-2 Dual Positive	Not-Evaluable
Female	17 Years of age to 21 Years of age	98	41.8% (41/98)	25.5% (25/98)	30.6% (30/98)	0.0% (0/98)	2.0% (2/98)
	More than 21 Years of age	503	54.3% (273/503)	16.9% (85/503)	25.8% (130/503)	0.8% (4/503)	2.2% (11/503)
	All	601	52.2% (314/601)	18.3% (110/601)	26.6% (160/601)	0.7% (4/601)	2.2% (13/601)
Male	17 Years of age to 21 Years of age	14	42.9% (6/14)	21.4% (3/14)	35.7% (5/14)	0.0% (0/14)	0.0% (0/14)
	More than 21 Years of age	66	68.2% (45/66)	10.6% (7/66)	18.2% (12/66)	1.5% (1/66)	1.5% (1/66)
	All	80	63.8% (51/80)	12.5% (10/80)	21.3% (17/80)	1.3% (1/80)	1.3% (1/80)
All		**681**	**53.6% (365/681)**	**17.6% (120/681)**	**26.0% (177/681)**	**0.7% (5/681)**	**0.7% (5/681)****

*Samples never tested due to temperature excursion or tested but "invalid" results obtained due to internal control failure, insufficient specimen volume, daily PC/NTC failure.
**Excluded from this table are 35 samples from patients less than 17 years of age and 2 samples where the age of the patient was not t available.

Hypothetical Prevalence	HSV-1		HSV-2	
	PPV*	NPV**	PPV*	NPV**
1.0%	35.3%	100.0%	30.9%	100.0%
2.0%	52.5%	99.9%	47.4%	99.9%
3.0%	62.6%	99.9%	57.7%	99.9%
5.0%	74.0%	99.9%	69.9%	99.8%
10.0%	85.7%	99.7%	83.1%	99.7%
15.0%	90.5%	99.5%	88.6%	99.5%
20.0%	93.1%	99.3%	91.7%	99.3%
30.0%	95.9%	98.9%	95.0%	98.8%
50.0%	98.2%	97.4%	97.8%	97.2%

*Positive Predictive Value (PPV) was calculated using:
(Sensitivity x Prevalence)/(Sensitivity x Prevalence + [1 - Specificity] x [1 - Prevalence]).
**Negative Predictive Value (NPV) was calculated using:
(Specificity x [1 - Prevalence])/([1 - Sensitivity] x Prevalence + Specificity x [1 - Prevalence]).

FRESH VS FROZEN STUDY

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.

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.

CONCLUSION

The conclusions drawn from the nonclinical and clinical tests demonstrate the device is as safe and effective as the legally marketed device identified above.