November 26, 2019

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

Re: K192376
Trade/Device Name: Simplexa VZV Swab Direct, Simplexa VZV 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 Codes: PGI, PMN
Dated: August 28, 2019
Received: August 30, 2019

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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; 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 https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). 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 (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Maria Ines Garcia, Ph.D.
Branch Chief
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Applicant	DiaSorin Molecular LLC. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Sharon Young Principal Regulatory Affairs Specialist tel 562.240.6680 fax 562.240.6530 Sharon Young@DiaSorin.com
Summary Date	November 18, 2019
Proprietary Name	Simplexa™ VZV Swab Direct and Simplexa™ VZV Positive Control Pack
US Product Codes/Names and Regulation Numbers	PGI / Herpes virus nucleic acid-based cutaneous and mucocutaneous lesion panel 21 CFR § 866.3309 PMN / Assayed external control material for microbiology nucleic acid amplification (NAT) assays 21 CFR § 866.3920
Classification	Class II
Predicate Device	Solana® HSV 1+2/VZV Assay (K162451)

Intended Use

Simplexa™ VZV Direct

The DiaSorin Molecular Simplexa™ VZV Swab Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection of varicella-zoster virus (VZV) DNA present in cutaneous and mucocutaneous lesion swabs from patients with signs and symptoms of VZV infection. This test is intended as an aid in the diagnosis of VZV infection. Negative results do not preclude VZV infection and should not be used as the sole basis for treatment or other patient management decisions.

Simplexa™ VZV Positive Control Pack

The Simplexa™ VZV Positive Control Pack is intended to be used as a control with the Simplexa™ VZV Direct kit and the Simplexa™ VZV Swab Direct kit on the LIAISON® MDX Instrument. It is not intended for use with other assays or systems.

Device Description

The Simplexa™ VZV Swab Direct assay is a real-time PCR system that enables the direct amplification and detection of VZV DNA from unprocessed cutaneous and mucocutaneous lesion swab specimens without nucleic acid extraction. The system consists of the Simplexa™ VZV Swab Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc (DAD) and associated accessories.

In the Simplexa™ VZV Swab Direct assay, fluorescent probes are used together with corresponding forward and reverse primers to amplify VZV and internal control targets. A well-conserved region of the VZV DNA polymerase gene is targeted to identify VZV DNA in the specimen. An internal control is used to detect PCR failure and/or inhibition.

Simplexa™ VZV Direct Kit

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ VZV Swab Direct Reaction Mix	MOL3656	REAG	C	RM	Black	24	1/24	50 µL

Simplexa™ VZV Direct Components and Descriptions

Kit Component	Contents				
Simplexa™ VZV Swab Direct Reaction Mix (RM)	DNA polymerase, buffer, dNTPs, template DNA (Internal Control), dye-labeled fluorescent probes and primers specific for detection of VZV Swab Direct and for the DNA Internal Control.				
	Target	**Probe Fluorophore (Dye)**	**Excitation (nm)**	**Emission (nm)**	**Targeted Gene**
	VZV	FAM	495	520	VZV DNA polymerase
	Internal Control DNA (IC)	Q670	644	670	N/A
Simplexa™ VZV Swab Direct Kit Barcode Card	Assay specific parameters and lot information.				

Simplexa™ VZV Positive Control Pack Component and Description

Component Name	REF	Description	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ VZV Direct Positive Control	MOL3661	Inactivated varicella-zoster virus	Red	10	1/10	50 µL

Materials Supplied Separately

Direct Amplification Disc Kit Direct Amplification Discs for use on the LIAISON® MDX

Comparison to Predicate Device

Comparison to Predicate Device	Predicate Device: Solana® HSV 1+2/VZV Assay (K162451)	Candidate Device: Simplexa™ VZV Direct and Simplexa™ VZV Positive Control Pack
Product Code	PGI	Same
Regulation Number	21 CFR 866.3309 – Herpes virus nucleic acid-based cutaneous and mucocutaneous lesion panel	Same
Organism Detected	Varicella-zoster virus	Same
Measurand	DNA from varicella-zoster virus	Same
Intended Use	The Solana® HSV 1+2/VZV Assay is an in vitro diagnostic test, using isothermal amplification technology (helicase-dependent amplification, HDA), for the 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 Solana® 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 Solana® HSV 1+2/VZV Assay is intended for use only with the Solana® instrument.	The DiaSorin Molecular Simplexa™ VZV Swab Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection of varicella-zoster virus (VZV) DNA present in cutaneous and mucocutaneous lesion swabs from patients with signs and symptoms of VZV infection. This test is intended as an aid in the diagnosis of VZV infection. Negative results do not preclude VZV infection and should not be used as the sole basis for treatment or other patient management decisions.
Automated System (Sample to Answer)	Yes	Yes
Instrumentation	Solana® Instrument	LIAISON® MDX

CLINICAL AGREEMENT

The performance of the Simplexa™ VZV Swab Direct assay was established in a clinical study that included three (3) cohorts based on sample status. Specifically, prospective and retrospective cutaneous and mucocutaneous swab samples from human patients with signs and symptoms of VZV infection, as well as contrived samples, were tested in the clinical agreement study.

Prospective Study

A total of four hundred fifty-two (452) cutaneous and mucocutaneous prospective specimens were collected from ten (10) collection sites across the USA during the clinical study (November 2018 – May 2019). The specimens were taken from anorectal, genital, nasal, ocular, oral, skin and urethral locations of the body. The age of the patients ranged from one (1) month to greater than 60 (>60) years of age. Of these specimens, sixty-two point four percent (62.4%) of the specimens were from female patients and thirty-seven point six percent (37.6%) of the specimens were from male patients. Ten (10) testing sites performed the Simplexa™ VZV Swab Direct assay on enrolled specimens and shipped the specimens to two (2) comparator testing sites to test against a three (3) part composite reference method (CRM). The three part CRM consisted of VZV direct stain fluorescent antibody (DSFA) and/or culture isolation with direct fluorescent antibody (DFA) and two (2) validated VZV polymerase chain reaction (PCR) assays followed by bi-directional sequencing. The comparator testing was performed by two (2) sites. One (1) testing site performed the VZV DSFA and/or culture isolation with DFA and another testing site conducted the two (2) validated VZV PCR assays testing followed by bi-directional sequencing. The results of the study are presented in Table 1a.

Table 1a. Simplexa™ VZV Swab Direct Prospective Agreement Results

Prospective Study	Composite Reference Method (CRM)				Total	Sensitivity 95% CI	Specificity 95% CI
	+		-				
	Simplexa™ VZV Swab Direct		Simplexa™ VZV Swab Direct				
	+	-	+	-			
Mucocutaneous	7	1[a]	0	171	179	87.5% (7/8) 52.9% - 97.8%	100.0% (171/171) 97.8% - 100.0%
Cutaneous	79	1[b]	3	162	245	98.8% (79/80) 93.3% - 99.8%	98.2% (162/165) 94.8% - 99.4%
Unknown	1	0	0	27	28	100.0% (1/1) 20.7% - 100.0%	100.0% (27/27) 87.5% - 100.0%
All	87	2	3	360	452	97.8% (87/89) 92.2% -99.4 %	99.2% 360/363) 97.6% - 99.7%

[a] The discordant negative mucocutaneous result is from an oral lesion sample. The sample was negative by the Simplexa™ VZV Swab Direct, DSFA/DFA and by the sites routine culture testing. The sample was positive by the two (2) PCR/Bi-directional sequencing assays.
[b] The discordant negative cutaneous result is from a skin lesion sample. The sample was negative by the Simplexa™ VZV Swab Direct, DSFA/DFA testing. The sample was positive by the 2 PCR/Bi-directional sequencing assays.
CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following Wilson Score method.

Retrospective Study

A total of sixty (60) cutaneous and mucocutaneous retrospective positive swab specimens in UTM were blinded and randomized with one hundred twenty (120) negative masked specimens prior to being tested by Simplexa™ VZV Swab Direct assay. The Composite Reference Method 2 (CRM 2) utilized a two (2) out of three (3) outcome from one (1) FDA Cleared NAAT PCR assay for VZV and two (2) validated VZV

PCR assays followed by bi-directional sequencing. The FDA Cleared NAAT was performed by one (1) external site. DiaSorin Molecular performed the Simplexa™ VZV Swab Direct testing and different DiaSorin Molecular operators performed the two (2) validated VZV PCR assays followed by bi-directional sequencing. The positive and negative percent agreement (PPA and NPA) results of the study are presented in Table 1b.

Table 1b. Simplexa™ VZV Swab Direct Retrospective Agreement Results

Retrospective Study	Composite Reference Method 2 (CRM 2)				Total	PPA 95% CI	NPA 95% CI
	+		-				
	Simplexa™ VZV Swab Direct		Simplexa™ VZV Swab Direct				
	+	-	+	-			
Mucocutaneous	9	1[c]	0	63	73	90.0% (9/10) 59.6% - 98.2%	100.0% (63/63) 94.3% - 100.0%
Cutaneous	52	0	1	54	107	100.0% (52/52) 93.1% - 100.0%	98.2% (54/55) 90.4% - 99.7%
All	61	1	1	117	180	98.4% (61/62) 91.4% - 99.7%	99.2% (117/118) 95.4% - 99.9%

[c] The discordant negative mucocutaneous result is from an oral lesion sample. The sample was negative by the Simplexa™ VZV Swab Direct and NAAT testing. The sample was positive by the two (2) PCR/Bi-directional sequencing assays.
CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following Wilson Score method.

Contrived Sample Study

A total of sixty (60) contrived positive specimens in individual negative UTM mucocutaneous swab matrix were blinded and randomized with sixty (60) masked negative UTM mucocutaneous specimens prior to being tested by Simplexa™ VZV Swab Direct assay. The results were compared with a two (2) out of three (3) outcome from one (1) FDA Cleared NAAT assay and two (2) validated VZV PCR assays followed by bi-directional sequencing (Composite Reference Method 2 or CRM 2). Of the sixty (60) contrived specimens, thirty (30) were spiked with VZV Ellen strain and thirty (30) were spiked with VZV 9939 strain at different known concentrations across the detection range. The results are presented in Table 1c.

Table 1c. Simplexa™ VZV Swab Direct Contrived Agreement Results

Contrived Mucocutaneous Samples	Composite Reference Method 2 (CRM 2)				Total	PPA 95% CI	NPA 95% CI
	+		-				
	Simplexa™ VZV Swab Direct		Simplexa™ VZV Swab Direct				
	+	-	+	-			
Ellen	30	0	0	0	30	100.0% (30/30) 88.6% - 100.0%	N/A
9939	30	0	0	0	30	100.0% (30/30) 88.6% - 100.0%	N/A
Negative	0	0	0	60	60	N/A	60/60 (100%) 94.0% -100.0%
All	60	0	0	60	120	100.0% (60/60) 94.0% - 100.0%	100.0% (60/60) 94.0% - 100.0%

CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following Wilson Score method.

REPRODUCIBILITY

Reproducibility for the Simplexa™ VZV Swab Direct assay was evaluated at three (3) investigative sites to assess the device's inter-site, inter/intra-day and inter/intra-assay reproducibility. Each of the laboratories tested a sample panel consisting of Simplexa™ VZV Swab Direct Positive Control, No Template Control, and four (4) contrived samples in negative matrix. Two (2) strains of VZV were used in the study, 9939 and Ellen. The four (4) contrived samples consisted of a low positive (LP) at 2X LoD and a medium positive (MP) at 4X LoD for each VZV strain. Each sample panel member was tested in triplicate per run, for two (2) runs per day by two (2) different operators at each site. Therefore, a total of ninety (90) replicates [three (3) replicates X two (2) runs X five (5) days X three (3) sites] were tested for each sample panel member. A total of six (6) LIAISON® MDX instruments [two (2) per site] were used to evaluate the reproducibility study. The combined results for all sites are presented in Table 2.

Table 2. Simplexa™ VZV Swab Direct Reproducibility

Summary of VZV Qualitative Results and VZV Ct Values ± SD (%CV)								
Sample	Site 1		Site 2		Site 4		All Sites	
	% Agreement With Expected Results	Detected Mean Ct ± SD (%CV)	% Agreement With Expected Results	Detected Mean Ct ± SD (%CV)	% Agreement With Expected Results	Detected Mean Ct ± SD (%CV)	% Agreement With Expected Results	Detected Mean Ct ± SD (%CV)
9939 LP	100.0% (30/30)	36.6 ± 1.12 (3.1%)	100.0% (30/30)	36.8 ± 0.68 (1.9%)	100.0% (30/30)	36.4 ± 0.83 (2.3%)	100.0% (90/90)	36.6 ± 0.9 (2.5%)
9939 MP	100.0% (30/30)	35.8 ± 0.86 (2.4%)	100.0% (30/30)	35.7 ± 0.54 (1.5%)	100.0% (30/30)	35.3 ± 0.78 (2.2%)	100.0% (90/90)	35.6 ± 0.76 (2.1%)
Ellen LP	100.0% (30/30)	35.4 ± 1.22 (3.4%)	100.0% (30/30)	34.5 ± 1.77 (5.1%)	100.0% (30/30)	35.0 ± 0.56 (1.6%)	100.0% (90/90)	35.0 ± 1.32 (3.8%)
Ellen MP	100.0% (30/30)	34.5 ± 0.65 (1.9%)	100.0% (30/30)	34.5 ± 0.47 (1.4%)	100.0% (30/30)	33.5 ± 1.3 (3.9%)	100.0% (90/90)	34.1 ± 0.99 (2.9%)
UTM (NTC)	0.0% (0/30)	0.0 ± 0.00 (N/A)	0.0% (0/30)	0.0 ± 0.00 (N/A)	0.0% (0/30)	0.0 ± 0.00 (N/A)	0.0% (0/90)	0.0 ± 0.00 (N/A)
PC	100.0% (30/30)	30.2 ± 0.74 (2.5%)	100.0% (30/30)	30.4 ± 0.59 (1.9%)	100.0% (30/30)	29.7 ± 0.86 (2.9%)	100.0% (90/90)	30.1 ± 0.79 (2.6%)

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The limit of detection (LoD) was determined for the Simplexa™ VZV Swab Direct assay using quantified stocks of two (2) VZV strains (Ellen and 9939) in a pool of cutaneous and mucocutaneous lesion swab sample types in UTM using forty-eight (48) replicates. The LoD was determined to be the lowest concentration that could be detected positive ≥95% of the time. The LoD results are presented in Table 3.

Table 3. Simplexa™ VZV Swab Direct Limit of Detection

VZV Strain	LoD ($TCID_{50}$/mL)	LoD (Copies /mL)
9939	0.77	800
Ellen	0.054	3500

ANALYTICAL VZV STRAIN REACTIVITY

Analytical VZV strain reactivity was evaluated with cutaneous and mucocutaneous lesion swab specimens with reference to LoD for the Simplexa™ VZV Swab Direct assay. Quantified viral material was spiked into negative matrix using a single dilution and assayed in triplicate. The Simplexa™ VZV Swab Direct assay was able to detect other strains of VZV. The results are presented in Table 4. In addition to the strains that were physically tested, in silico BLAST analysis demonstrated that the assay is expected to detect at least one hundred seventy-eight (178) additional VZV strains.

Table 4. Simplexa™ VZV Swab Direct Analytical Reactivity With VZV Strains

VZV Strain	Concentration ($TCID_{50}$/mL)	Qualitative Result (# Detected/# Tested)
VZV Strain 82	0.82	3/3
VZV Strain 275	0.82	3/3
VZV Strain 1700	2.47	3/3
VZV Isolate A	0.82	3/3
VZV Isolate B	0.82	3/3
VZV Isolate D	0.82	3/3

CROSS-REACTIVITY (Analytical Specificity)

The Simplexa™ VZV Swab Direct assay's analytical specificity was evaluated by testing the ability of the assay to exclusively identify VZV with no cross-reactivity to organisms that are closely related, or cause similar clinical symptoms or that could be found in cutaneous and mucocutaneous lesion swab specimens. Analytical specificity/cross-reactivity was tested with ninety-nine (99) different bacteria, viruses, parasites and fungi and assayed in triplicate. No cross-reactivity was observed with the ninety-nine (99) organisms. The organisms and the concentration at which these were tested are presented in Table 5.

Table 5. Simplexa™ VZV Swab Direct Cross-Reactivity

Organism	Tested Concentration	Organism	Tested Concentration
Acholeplasma laidlawi (genomic DNA)	1 x 10^6 copies/mL	Human genomic DNA	1 x 10^6 copies/mL
Acinetobacter calcoaceticus	1 x 10^6 CFU/mL	Human metapneumovirus A1	1 x 10^5 $TCID_{50}$/mL
Acinetobacter lwoffi	1 x 10^6 CFU/mL	Human Papilloma Virus 18	1 x 10^5 copies/mL
Adenovirus 7A	1 x 10^5 $TCID_{50}$/mL	Influenza A/California/7/2009	1 x 10^5 $TCID_{50}$/mL
Bacteroides fragilis	1 x 10^6 CFU/mL	Influenza B/Florida/02/2006	1 x 10^5 $TCID_{50}$/mL
Bordetella bronchiseptica	1 x 10^6 CFU/mL	*Klebsiella pneumoniae*	1 x 10^6 CFU/mL
Bordetella pertussis	1 x 10^6 CFU/mL	*Lactobacillus acidophilus*	1 x 10^6 CFU/mL
Borrelia burgdorferi (genomic DNA)	1 x 10^6 copies/mL	*Legionella pneumophila*	1 x 10^6 CFU/mL
Candida albicans	1 x 10^6 CFU/mL	Measles virus	1 x 10^5 $TCID_{50}$/mL
Candida glabrata	1 x 10^6 CFU/mL	*Mobiluncus curtisii*	1 x 10^6 CFU/mL
Candida guilliermondii	1 x 10^6 CFU/mL	*Mobiluncus mulieris*	1 x 10^6 CFU/mL
Candida krusei	1 x 10^6 CFU/mL	*Moraxella cartarrhalis*	1 x 10^6 CFU/mL
Candida lusitaniae	1 x 10^6 CFU/mL	Mumps virus	1 x 10^5 $TCID_{50}$/mL
Candida parapsilosis	1 x 10^6 CFU/mL	*Mycoplasma genitalium*	1 x 10^6 CCU/mL
Candida tropicalis	1 x 10^6 CFU/mL	*Mycoplasma hominis*	1 x 10^6 CCU/mL
Chlamydia trachomatis	1 x 10^6 IFU/mL	*Mycoplasma hyorhinis*	1 x 10^6 CCU/mL
Chlamydophila pneumoniae	1 x 10^6 IFU/mL	*Mycoplasma orale*	1 x 10^6 CCU/mL
Clostridium difficile	1 x 10^6 CFU/mL	*Mycoplasma pneumoniae*	1 x 10^6 CCU/mL
Clostridium perfringens	1 x 10^6 CFU/mL	*Mycoplasma salivarium*	1 x 10^6 CCU/mL
Clostridium sordellii	1 x 10^6 CFU/mL	*Neisseria gonorrhoeae*	1 x 10^6 CFU/mL
Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	*Neisseria meningitidis*	1 x 10^6 CFU/mL
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	Parainfluenza Type 1	1 x 10^5 $TCID_{50}$/mL
Corynebacterium genitalium	1 x 10^6 CFU/mL	Parainfluenza Type 2	1 x 10^5 $TCID_{50}$/mL
Coxsackievirus B1	1 x 10^5 $TCID_{50}$/mL	Parainfluenza Type 3	1 x 10^5 $TCID_{50}$/mL
Coxsackievirus B4	1 x 10^5 $TCID_{50}$/mL	Parainfluenza Type 4	1 x 10^5 $TCID_{50}$/mL
Cytomegalovirus (AD169 strain)	1 x 10^5 $TCID_{50}$/mL	*Prevotella melaninogenica*	1 x 10^6 CFU/mL
Cytomegalovirus (Towne strain)	1 x 10^5 $TCID_{50}$/mL	*Proteus mirabilis*	1 x 10^6 CFU/mL
Echovirus 11	1 x 10^5 $TCID_{50}$/mL	*Proteus vulgaris*	1 x 10^6 CFU/mL
Enterobacter cloacae	1 x 10^6 CFU/mL	*Pseudomonas aeruginosa*	1 x 10^6 CFU/mL
Enterococcus faecalis vanB	1 x 10^6 CFU/mL	RSV A Long	1 x 10^5 $TCID_{50}$/mL

Organism	Tested Concentration	Organism	Tested Concentration
Enterococcus faecium	1 x 10^6 CFU/mL	RSV B Washington	1 x 10^5 $TCID_{50}$/mL
Enterovirus 70	1 x 10^5 $TCID_{50}$/mL	Rubella Virus	1 x 10^5 $TCID_{50}$/mL
Enterovirus 71	1 x 10^5 $TCID_{50}$/mL	*Salmonella enteritidis* (genomic DNA)	1 x 10^6 copies/mL
Epstein Barr Virus (B95-8 strain)	1 x 10^5 copies/mL	*Salmonella typhimurium*	1 x 10^6 CFU/mL
Escherichia coli O15:H7	1 x 10^6 CFU/mL	*Serratia marcescens*	1 x 10^6 CFU/mL
Fusobacterium nucleatum	1 x 10^6 CFU/mL	Simian Virus type 40	1 x 10^5 $TCID_{50}$/mL
Gardnerella vaginalis	1 x 10^6 CFU/mL	*Staphylococcus aureus* (MRSA), ATCC 700699	1 x 10^6 CFU/mL
Haemophilus ducreyi	1 x 10^6 CFU/mL	*Staphylococcus aureus* (MRSA), COL	1 x 10^6 CFU/mL
Haemophilus influenza type A	1 x 10^6 CFU/mL	*Staphylococcus epidermidis* (MRSE), ATCC 29887	1 x 10^6 CFU/mL
Hepatitis A virus	1 x 10^6 $TCID_{50}$/mL	*Staphylococcus saprophyticus*	1 x 10^6 CFU/mL
Hepatitis B virus	1 x 10^5 IU/mL	*Streptococcus agalactiae*	1 x 10^6 CFU/mL
Hepatitis C virus	1 x 10^5 IU/mL	*Streptococcus mitis*	1 x 10^6 CFU/mL
HHV-6 (Z29 strain)	1 x 10^5 copies/mL	*Streptococcus mutans*	1 x 10^6 CFU/mL
HHV-6A	1 x 10^5 copies/mL	*Streptococcus pneumoniae*	1 x 10^6 CFU/mL
HHV-7 SB	1 x 10^5 $TCID_{50}$/mL	*Streptococcus pyogenes*, M1	1 x 10^6 CFU/mL
HHV-8	1 x 10^5 copies/mL	*Streptococcus salivarius*	1 x 10^6 CFU/mL
HIV-1 IIIB	1 x 10^5 $TCID_{50}$/mL	*Toxoplasma gondii*	1 x 10^6 tachyzoites/mL
HIV-2 NIHZ	1 x 10^5 $TCID_{50}$/mL	*Trichomonas vaginalis*	1 x 10^6 trophozoites/mL
HSV-1 (McIntyre strain)	1 x 10^5 $TCID_{50}$/mL	*Ureaplasma urealyticum*	1 x 10^6 CCU/mL
HSV-2 (G strain)	1 x 10^5 $TCID_{50}$/mL		

Note: *Bacteroides ureolyticus*, Hepatitis D virus, *Treponema pallidum* and *Tropheryma whipplei* were tested using *in silico* NCBI BLAST analysis due to unavailability of the organism. No cross-reactivity was found.

INHIBITION BY OTHER MICROORGANISMS

The Simplexa™ VZV Swab Direct assay was evaluated by testing the ability to identify VZV virus (Ellen and 9939 strains) when other potential inhibitory organisms are present. A panel of ninety-nine (99) potentialy inhibitory organisms were individually spiked into pooled cutaneous and mucocutaneous swab matrix containing a low concentration of VZV at approximately 2X LoD and tested in triplicate. Table 6 below references the microorganisms and their respective tested concentration. No inhibition was observed for the detection of either VZV Ellen or 9939 strains as shown.

Table 6. Simplexa™ VZV Swab Direct Microbial Inhibition

Organism	Tested Concentration	Organism	Tested Concentration
Acholeplasma laidlawi (genomic DNA)	1 x 10^6 copies/mL	Human genomic DNA	1 x 10^6 copies/mL
Acinetobacter calcoaceticus	1 x 10^6 CFU/mL	Human metapneumovirus A1	1 x 10^5 $TCID_{50}$/mL
Acinetobacter lwoffi	1 x 10^6 CFU/mL	Human papilloma virus 18	1 x 10^5 copies/mL
Adenovirus 7A	1 x 10^5 $TCID_{50}$/mL	Influenza A/California/7/2009	1 x 10^5 $TCID_{50}$/mL
Bacteroides fragilis	1 x 10^6 CFU/mL	Influenza B/Florida/02/2006	1 x 10^5 $TCID_{50}$/mL
Bordetella bronchiseptica	1 x 10^6 CFU/mL	*Klebsiella pneumoniae*	1 x 10^6 CFU/mL
Bordetella pertussis	1 x 10^6 CFU/mL	*Lactobacillus acidophilus*	1 x 10^6 CFU/mL
Borrelia burgdorferi (genomic DNA)	1 x 10^6 copies/mL	*Legionella pneumophila*	1 x 10^6 CFU/mL
Candida albicans	1 x 10^6 CFU/mL	Measles virus	1 x 10^5 $TCID_{50}$/mL
Candida glabrata	1 x 10^6 CFU/mL	*Mobiluncus curtisii*	1 x 10^6 CFU/mL
Candida guilliermondii	1 x 10^6 CFU/mL	*Mobiluncus mulieris*	1 x 10^6 CFU/mL
Candida krusei	1 x 10^6 CFU/mL	*Moraxella cartarrhalis*	1 x 10^6 CFU/mL
Candida lusitaniae	1 x 10^6 CFU/mL	Mumps virus	1 x 10^5 $TCID_{50}$/mL
Candida parapsilosis	1 x 10^6 CFU/mL	*Mycoplasma genitalium*	1 x 10^6 CCU/mL
Candida tropicalis	1 x 10^6 CFU/mL	*Mycoplasma hominis*	1 x 10^6 CCU/mL
Chlamydia trachomatis	1 x 10^6 IFU/mL	*Mycoplasma hyorhinis*	1 x 10^6 CCU/mL
Chlamydophila pneumoniae	1 x 10^6 IFU/mL	*Mycoplasma orale*	1 x 10^6 CCU/mL
Clostridium difficile	1 x 10^6 CFU/mL	*Mycoplasma pneumoniae*	1 x 10^6 CCU/mL
Clostridium perfringens	1 x 10^6 CFU/mL	*Mycoplasma salivarium*	1 x 10^6 CCU/mL
Clostridium sordellii	1 x 10^6 CFU/mL	*Neisseria gonorrhoeae*	1 x 10^6 CFU/mL
Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	*Neisseria meningitidis*	1 x 10^6 CFU/mL
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	Parainfluenza Type 1	1 x 10^5 $TCID_{50}$/mL
Corynebacterium genitalium	1 x 10^6 CFU/mL	Parainfluenza Type 2	1 x 10^5 $TCID_{50}$/mL
Coxsackievirus B1	1 x 10^5 $TCID_{50}$/mL	Parainfluenza Type 3	1 x 10^5 $TCID_{50}$/mL
Coxsackievirus B4	1 x 10^5 $TCID_{50}$/mL	Parainfluenza Type 4	1 x 10^5 $TCID_{50}$/mL
Cytomegalovirus (AD169 strain)	1 x 10^5 $TCID_{50}$/mL	*Prevotella melaninogenica*	1 x 10^6 CFU/mL
Cytomegalovirus (Towne strain)	1 x 10^5 $TCID_{50}$/mL	*Proteus mirabilis*	1 x 10^6 CFU/mL
Echovirus 11	1 x 10^5 $TCID_{50}$/mL	*Proteus vulgaris*	1 x 10^6 CFU/mL
Enterobacter cloacae	1 x 10^6 CFU/mL	*Pseudomonas aeruginosa*	1 x 10^6 CFU/mL
Enterococcus faecalis vanB	1 x 10^6 CFU/mL	RSV A Long	1 x 10^5 $TCID_{50}$/mL

Organism	Tested Concentration	Organism	Tested Concentration
Enterococcus faecium	1 x 10^6 CFU/mL	RSV B Washington	1 x 10^5 $TCID_{50}$/mL
Enterovirus 70	1 x 10^5 $TCID_{50}$/mL	Rubella Virus	1 x 10^5 $TCID_{50}$/mL
Enterovirus 71	1 x 10^5 $TCID_{50}$/mL	*Salmonella enteritidis* (genomic DNA)	1 x 10^6 copies/mL
Epstein Barr Virus (B95-8 strain)	1 x 10^5 copies/mL	*Salmonella typhimurium*	1 x 10^6 CFU/mL
Escherichia coli O15:H7	1 x 10^6 CFU/mL	*Serratia marcescens*	1 x 10^6 CFU/mL
Fusobacterium nucleatum	1 x 10^6 CFU/mL	Simian Virus type 40	1 x 10^5 $TCID_{50}$/mL
Gardnerella vaginalis	1 x 10^6 CFU/mL	*Staphylococcus aureus* (MRSA), ATCC 700699	1 x 10^6 CFU/mL
Haemophilus ducreyi	1 x 10^6 CFU/mL	*Staphylococcus aureus* (MRSA), COL	1 x 10^6 CFU/mL
Haemophilus influenza type A	1 x 10^6 CFU/mL	*Staphylococcus epidermidis* (MRSE), ATCC 29887	1 x 10^6 CFU/mL
Hepatitis A virus	1 x 10^6 $TCID_{50}$/mL	*Staphylococcus saprophyticus*	1 x 10^6 CFU/mL
Hepatitis B virus	1 x 10^5 IU/mL	*Streptococcus agalactiae*	1 x 10^6 CFU/mL
Hepatitis C virus	1 x 10^5 IU/mL	*Streptococcus mitis*	1 x 10^6 CFU/mL
HHV-6 (Z29 strain)	1 x 10^5 copies/mL	*Streptococcus mutans*	1 x 10^6 CFU/mL
HHV-6A	1 x 10^5 copies/mL	*Streptococcus pneumoniae*	1 x 10^6 CFU/mL
HHV-7 SB	1 x 10^5 $TCID_{50}$/mL	*Streptococcus pyogenes*, M1	1 x 10^6 CFU/mL
HHV-8	1 x 10^5 copies/mL	*Streptococcus salivarius*	1 x 10^6 CFU/mL
HIV-1 IIIB	1 x 10^5 $TCID_{50}$/mL	*Toxoplasma gondii*	1 x 10^6 tachyzoites/mL
HIV-2 NIHZ	1 x 10^5 $TCID_{50}$/mL	*Trichomonas vaginalis*	1 x 10^6 trophozoites/mL
HSV-1 (McIntyre strain)	1 x 10^5 $TCID_{50}$/mL	*Ureaplasma urealyticum*	1 x 10^6 CCU/mL
HSV-2 (G strain)	1 x 10^5 $TCID_{50}$/mL		

Note: *Bacteroides ureolyticus*, Hepatitis D virus, *Treponema pallidum* and *Tropheryma whipplei* were tested using *in silico* NCBI BLAST analysis due to unavailability of the organism. No interference was found.

INTERFERENCE

The performance of the Simplexa™ VZV Swab Direct assay was evaluated with potentially interfering substances. The tested concentrations of the potentially interfering endogenous and exogenous substances are indicated in the table below (Table 7). A total of forty-five (45) potential interfering substances were individually spiked into a pooled cutaneous and mucocutaneous swab matrix containing a low concentration of VZV at approximately 2X LoD and tested in triplicate. No interference was observed as presented in Table 7 for Ellen and 9939 Strains.

Table 7. Simplexa™ VZV Swab Direct Interference

Potentially Interfering Substance.	VZV Strain	Active Ingredient	Tested Concentration	# Detected /# Tested
Abreva	9936	Docosanol 10%	7% (w/v)	3/3
	Ellen			3/3
Acetaminophen	9936	N/A	7% (w/v)	3/3
	Ellen			3/3
Acyclovir	9936	N/A	10 mg/mL	3/3
	Ellen			3/3
Albumin	9936	N/A	10 mg/mL	3/3
	Ellen			3/3
Balneol lotion	9936	Buffers, emulsifiers, PEG, water, mineral oil, lanolin oil, preservatives	7% (v/v)	3/3
	Ellen			3/3
Carmex	9936	Camphor, 1.7%; Menthol, 0.7%	10% (w/v)	3/3
	Ellen			3/3
Casein	9936	N/A	10 mg/mL	3/3
	Ellen			3/3
Chlor-Trimeton	9936	Chlorpheniramine maleate	5 mg/mL	3/3
	Ellen			3/3
Cidofovir	9936	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Clotrimazole Vaginal Cream	9936	Clotrimazole	7% (w/v)	3/3
	Ellen		3.5% (w/v)	3/3
Cold-Eeze	9936	Zincum Gluconicum 2X	10% (w/v)	3/3
			5% (w/v)	3/3
	9936		2.5% (w/v)	3/3
Cornstarch	9936	N/A	1.25 mg/mL	3/3
	Ellen			3/3
Denavir	9936	N/A	2.5 mg/mL	3/3
	Ellen			3/3

Potentially Interfering Substance.	VZV Strain	Active Ingredient	Tested Concentration	# Detected /# Tested
Desitin	9936	Zinc Oxide, 40%	7% (w/v)	3/3
			3.5% (w/v)	3/3
	Ellen		3.5% (w/v)	3/3
Dextromethorphan hydrobromide (Robitussin-DM)	9936	Dextromethorphan hydrobromide	10 mg/mL	3/3
	Ellen			3/3
Douche	9936	N/A	7% (v/v)	3/3
	Ellen			3/3
Famciclovir	9936	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Feces	9936	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Foscarnet	9936	N/A	1.25 mg/mL	3/3
	Ellen			3/3
Glucose	9936	N/A	11 mg/mL	3/3
	Ellen			3/3
Gynol II contraceptive jelly	9936	Nonoxynol-9 (3%)	7% (w/v)	3/3
	Ellen			3/3
Human genomic DNA	9936	N/A	20 µg/mL	3/3
	Ellen			3/3
Immunoglobulin	9936	N/A	10 mg/mL	3/3
	Ellen			3/3
KY Jelly	9936	N/A	10 mg/mL	3/3
	Ellen		5% (w/v)	3/3
Lactate	9936	N/A	2.2 mg/mL	3/3
	Ellen			3/3
Lanacane	9936	Benzethonium chloride, 0.2%; Benzocaine, 20%	7% (v/v)	3/3
	Ellen			3/3
Lip-Clear Lysine	9936	Zinc Oxide, 1.2%	7% (w/v)	3/3

Potentially Interfering Substance.	VZV Strain	Active Ingredient	Tested Concentration	# Detected /# Tested
	Ellen		3.5% (w/v)	3/3
Miconazole 1	9936	Miconazole nitrate, 26%	10% (w/v)	3/3
	Ellen			3/3
Miconazole 3	9936	Miconazole nitrate, 2%	10% (w/v)	3/3
	Ellen			3/3
Monistat 1 insert	9936	Miconazole nitrate, 1200 mg	7% (w/v)	3/3
	Ellen			3/3
Monistat 3 cream	9936	Miconazole nitrate 2%	7% (w/v)	3/3
	Ellen			3/3
Mouthwash (Listerine)	9936	Eucalyptol, 0.092%; Menthol, 0.042%; Methyl salicylate, 0.060%; Thymol, 0.064%	7% (v/v)	3/3
	Ellen			3/3
Mucin	9936	N/A	5% (w/v)	3/3
	Ellen			3/3
Preparation H	9936	N/A	10% (w/v)	3/3
	Ellen			3/3
Releev	9936	N/A	10% (w/v)	3/3
	Ellen			3/3
Seminal Fluid	9936	N/A	10% (v/v)	3/3
	Ellen			3/3
Tioconazole 1	9936	N/A	10% (w/v)	3/3
	Ellen			3/3
Toothpaste (Colgate)	9936	Sodium fluoride, 0.243%	7% (w/v)	3/3
	Ellen			3/3
Urine	9936	N/A	10% (v/v)	3/3
	Ellen			3/3
Vagisil creme	9936	Benzocaine (5%), Resorcinol (2%)	7% (w/v)	3/3
	Ellen			3/3
Valacyclovir	9936	N/A	2.5 mg/mL	3/3

Potentially Interfering Substance.	VZV Strain	Active Ingredient	Tested Concentration	# Detected /# Tested
	Ellen			3/3
Valgancyclovir	9936	N/A	2.5 mg/mL	3/3
	Ellen			3/3
White blood cells	9936	N/A	$5.5x10^7$ cells/mL	3/3
	Ellen			3/3
Whole Blood in EDTA	9936	N/A	10% (v/v)	3/3
	Ellen			3/3
YeastGard suppositories	9936	*Candida albicans* 27X HPU (*Candida albicans*), *Candida parapsilosis* 27X HPUS (*Candida parapsilosis*), Pulsatilla 27X HPUS (Meadow Anemone)	7% (w/v)	3/3
	Ellen			3/3

CARRY-OVER CONTAMINATION

The amplification carry-over for the Simplexa™ assays including the Simplexa™ VZV Swab Direct was assessed from the Simplexa™ Flu A/B & RSV Direct viral assay. The study can be applied to the Simplexa™ VZV Swab Direct assay as the study is not analyte specific. In the Simplexa™ Flu A/B & RSV Direct, 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.

CONCLUSION

From the above analytical and comparative testing results of the Simplexa™ VZV Swab Direct assay it is concluded that it is substantially equivalent to the FDA cleared device Solana® HSV 1+2/VZV Assay (K162451).