May 13, 2019

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

Re: K190219
Trade/Device Name: Simplexa VZV Direct, Simplexa VZV Positive Control Pack
Regulation Number: 21 CFR 866.3970
Regulation Name: Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid
Regulatory Class: Class II
Product Code: PLO
Dated: January 30, 2019
Received: February 4, 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/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); 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 http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

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 yours,

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)*
K190219

Device Name
Simplexa VZV Direct, Simplexa VZV Positive Control Pack

Indications for Use *(Describe)*
The DiaSorin Molecular Simplexa™ VZV Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection of varicella-zoster virus (VZV) DNA in cerebrospinal fluid (CSF) from patients signs and/or symptoms of meningitis and/or encephalitis. This test is intended as an aid in the diagnosis of VZV infections of the central nervous system (CNS).
Negative results do not preclude VZV infection and should not be used as the sole basis for treatment or other patient management decisions.
The assay is not intended for use as a donor screening test. The assay is for professional use only.

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 Principal Regulatory Affairs Specialist tel 562.240.6680 fax 562.240.6529 Sharon.Young@DiaSorin.com
Summary Date	May 3, 2019
Proprietary Name	Simplexa™ VZV Direct and Simplexa™ VZV Positive Control Pack
US Product Codes/Names and Regulation Numbers	PLO / Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid / 21 CFR § 866.3970 OOI / Real Time Nucleic Acid Amplification System / 21 CFR § 862.2570
Classification	Class II
Predicate Devices	BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel K160462

Intended Use

Simplexa™ VZV Direct REF MOL3650

The DiaSorin Molecular Simplexa™ VZV Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection of varicella-zoster virus (VZV) DNA in cerebrospinal fluid (CSF) from patients with signs and/or symptoms of meningitis and/or encephalitis. This test is intended as an aid in the diagnosis of VZV infections of the central nervous system (CNS).

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

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

Simplexa™ VZV Positive Control Pack REF MOL3660

The Simplexa™ VZV Positive Control Pack is intended to be used as a control with the Simplexa™ VZV Direct kit.

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

Device Description

The Simplexa™ VZV Direct assay is a real-time polymerase chain reaction (PCR) system that enables the direct amplification and detection of VZV DNA from unprocessed cerebral spinal fluid (CSF) specimens without nucleic acid extraction. The system consists of the Simplexa™ VZV Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories. In the Simplexa™ VZV 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 REF MOL3650

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ VZV Direct Reaction Mix	MOL3651	REAG	C	RM	Purple	24	1/24	50 µL

Simplexa™ VZV Direct Components and Descriptions

<table>
<tr><th>Kit Component</th><th>Contents</th></tr>
<tr><td>Simplexa™ VZV Direct Reaction Mix (RM)</td><td>
DNA polymerase, buffer, dNTPs, template DNA (Internal Control), dye-labeled fluorescent probes and primers specific for detection of VZV 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>VZV</td><td>FAM</td><td>495</td><td>520</td><td>VZV DNA polymera</td></tr>
<tr><td>Internal Control DNA</td><td>Q670</td><td>644</td><td>670</td><td>DNA (IC)</td></tr>
</table>
</td></tr>
<tr><td>Simplexa™ VZV Direct Kit Barcode Card</td><td>Assay specific parameters and lot information.</td></tr>
</table>

Simplexa™ VZV Positive Control Pack REF MOL3660
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 (REF MOL1455) Direct Amplification Discs for use on the LIAISON® MDX

Comparison to Predicate Device

Comparison to Predicate Device	Predicate Device: BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel K160462	Candidate Device: Simplexa™ VZV Direct and Simplexa™ VZV Positive Control Pack
Product Code	PLO	Same
Regulation Number	21 CFR 866.3970 – Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid.	Same
Organism Detected	varicella zoster virus	Same
Measurand	DNA from varicella zoster virus	Same
Intended Use	The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with FilmArray and FilmArray 2.0 systems. The FilmArray ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis. The following organisms are identified using the FilmArray ME Panel: Bacteria: Escherichia coli K1 Haemophilus influenzae Listeria monocytogenes Neisseria meningitidis (encapsulated) Streptococcus agalactiae Streptococcus pneumoniae Viruses: Cytomegalovirus Enterovirus Herpes simplex virus 1 Herpes simplex virus 2 Human herpesvirus 6 Human parechovirus Varicella zoster virus Yeast: Cryptococcus neoformans/gattii The FilmArray ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or encephalitis and results are meant to be used in conjunction with other clinical, epidemiological, and laboratory data. Results from the FilmArray ME Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management	Simplexa™ VZV Direct REF MOL3650 The DiaSorin Molecular Simplexa™ VZV Direct assay is intended for use on the LIAISON® MDX instrument for the qualitative detection of varicella-zoster virus (VZV) DNA in cerebrospinal fluid (CSF) from patients with signs and/or symptoms of meningitis and/or encephalitis. This test is intended as an aid in the diagnosis of VZV infections of the central nervous system (CNS). Negative results do not preclude VZV infection and should not be used as the sole basis for treatment or other patient management decisions. The assay is not intended for use as a donor screening test. The assay is for professional use only. Simplexa™ VZV Positive Control Pack REF MOL3660 The Simplexa™ VZV Positive Control Pack is intended to be used as a control with the Simplexa™ VZV Direct kit. This control is not intended for use with other assays or systems.

Comparison to Predicate Device	Predicate Device: BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel K160462	Candidate Device: Simplexa™ VZV Direct and Simplexa™ VZV Positive Control Pack
	decisions. Positive results do not rule out co-infection with organisms not included in the FilmArray ME Panel. The agent detected may not be the definite cause of the disease. Negative results do not preclude central nervous system (CNS) infection. Not all agents of CNS infection are detected by this test and sensitivity in clinical use may differ from that described in the package insert. The FilmArray ME Panel is not intended for testing of specimens collected from indwelling CNS medical devices. The FilmArray ME Panel is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing.	
Automated System (Sample to Answer)	Yes	Yes
Instrumentation	FilmArray or FilmArray 2.0 system	LIAISON® MDX

CLINICAL AGREEMENT
A total of six hundred thirty-seven (637) clinical samples, meeting inclusion criteria were obtained from prospective and prospectively banked collections from eight (8) collection sites from February 2018 to November 2018. The study was conducted using remnant cerebrospinal fluid (CSF) samples from human patients suspected of VZV infection of the central nervous system (CNS).

Due to the low prevalence of VZV infection in CSF samples, two hundred forty (240) contrived samples were included to supplement the number of positive samples tested. These samples were contrived across the clinical range of the Simplexa™ VZV Direct assay and thirty percent (30%) of the samples were contrived at approximately 2X LoD. The samples were stored at 2-8 °C for up to seven (7) days post collection and at <-70 °C thereafter. All Simplexa™ VZV Direct testing was performed at the testing sites.

Simplexa™ VZV Direct results were compared to results from a composite reference method. The composite reference method consisted of two (2) validated real-time PCR assays followed by confirmation of positive PCR amplification products with bi-directional sequencing. Samples were characterized as positive if one (1) or both PCR assays were positive and confirmed by bi-directional sequencing. Samples were characterized as negative if both PCR assays were negative. Samples were tested on Simplexa™ VZV Direct at the collection sites and the composite reference method was performed at DiaSorin Molecular, Cypress, CA.

Table 1 shows the Clinical Agreement of the Simplexa™ VZV Direct as compared to the composite reference method of two (2) validated real-time PCR assays followed by confirmation of positive PCR amplification products with bi-directional sequencing.

Table 1. Prospective Clinical Agreement Results for Simplexa™ VZV Direct vs Composite Reference Method

Simplexa™ VZV Direct Results	Composite PCR/Sequencing		
	Detected	**Not Detected**	**Total**
Detected	12	2[a]	14
Not Detected	0	623	623
Total	12	625	637
	PPA 100.0% (12/12) 95% CI: 75.7% to 100.0%	**NPA** 99.7% (623/625) 95% CI: 98.8% to 99.9%	

[a] One (1) of two (2) discordant results were positive with an alternate NAAT used by collection site during routine testing.

Due to the low prevalence of VZV infection in CSF samples, contrived positive samples were tested to supplement the number of positive samples tested. For each of the two strains, VZV Ellen and VZV 9939 60 samples were contrived across the clinical range of the Simplexa VZV Direct assay with 60% of the samples contrived as low positive samples close to the LoD of the test. Samples were tested at four clinical sites in a blinded manner, randomized with VZV negative samples. The samples were stored at 2-8 °C for up to 7 days post collection and at <-70 °C thereafter. Simplexa VZV Direct test results for contrived samples were compared to the same Composite Reference Method described for the prospective clinical study above. The contrived sample study demonstrated a positive percent agreement of 100% (120/120) with a 95% Confidence Interval of 96.9-100.0%.

REPRODUCIBILITY

Reproducibility for the Simplexa™ VZV Direct assay was evaluated. Three (3) investigative sites assessed the device's inter-site, inter-day and inter/intra-assay reproducibility. Each of the laboratories tested Simplexa™ VZV Direct Positive Control, No Template Control (Synthetic CSF), 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) and medium positive (MP) for each VZV strain. The assays were performed in triplicate on five (5) different days. Each site had two (2) operators who each assayed the entire sample panel and Positive Control once per day, for a total of two (2) sets of data per day on a total of six (6) LIAISON® MDX instruments. The combined results for all sites are presented in Tables 2 and 3. The results show the reproducibility of the Simplexa™ VZV Direct % CV to range between 0.5-4.6.

Table 2. Simplexa™ VZV Direct Reproducibility – VZV (FAM)

Sample VZV Strain (FAM)	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		
9939 – LP	100.0% (30/30)	35.8	4.6	100.0% (30/30)	35.7	3.6	100.0% (30/30)	36.1	4.2	100.0% (90/90)	95.9% to 100.0%
9939 – MP	100.0% (30/30)	35.3	2.2	100.0% (30/30)	34.6	2.4	100.0% (30/30)	35.0	2.3	100.0% (90/90)	95.9% to 100.0%
Ellen – LP	100.0% (30/30)	36.7	2.6	100.0% (30/30)	36.9	2.2	100.0% (30/30)	36.9	1.6	100.0% (90/90)	95.9% to 100.0%
Ellen – MP	100.0% (30/30)	35.6	1.6	100.0% (30/30)	35.6	1.0	100.0% (30/30)	35.6	1.1	100.0% (90/90)	95.9% to 100.0%
Positive Control	100.0% (30/30)	30.3	1.1	100.0% (30/30)	29.8	1.2	100.0% (30/30)	30.7	0.9	100.0% (90/90)	95.9% to 100.0%
NTC	100.0% (30/30)	0.0	N/A	100.0% (30/30)	0.0	N/A	100.0% (30/30)	0.0	N/A	100.0% (90/90)	95.9% to 100.0%
Total Agreement	**100.0% (180/180) 95% CI: 97.9% to 100.0%**			**100.0% (180/180) 95% CI: 97.9% to 100.0%**			**100.0% (180/180) 95% CI: 97.9% to 100.0%**			**100.0% (540/540) 95% CI: 99.3% to 100.0%**	

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) was determined for the Simplexa™ VZV Direct assay using quantified stocks of two (2) VZV strains (Ellen and 9939) serially diluted into negative human CSF matrix. The LoD was determined to be the lowest concentration that could be detected positive $\geq$ 95% of the time.

Table 3. Simplexa™ VZV Direct Limit of Detection

VZV strain	Concentration	
	$TCID_{50}$/mL	copies/mL
9939	2.03 $TCID_{50}$/mL	1,614
Ellen	0.001 $TCID_{50}$/mL	1,505

ANALYTICAL REACTIVITY/CROSS REACTIVITY

Analytical Reactivity

The analytical reactivity of the Simplexa™ VZV Direct assay was evaluated using different strains of VZV that were not used in the determination of the limit of detection (LoD) for the assay. Quantified viral material was spiked into negative CSF using a single dilution and assayed in triplicate. The Simplexa™ VZV Direct assay was able to detect other strains of VZV at 2X LoD. The results are presented in Table 4. In addition, *in silico* BLAST analysis indicated that the assay should detect at least one hundred seventy-eight (178) additional VZV strains.

Table 4. Simplexa™ VZV Direct Analytical Reactivity

VZV Strain	Agreement with Expected Results (#Detected/#Total)
VZV Strain 82	3/3
VZV Strain 275	3/3
VZV Strain 1700	3/3
VZV Isolate A	3/3
VZV Isolate B	3/3

Cross-Reactivity (Analytical Specificity)

The Simplexa™ VZV Direct assay's analytical specificity was evaluated by testing the ability of the assay to exclusively identify VZV virus with no cross-reactivity to organisms that are closely related, or cause similar clinical symptoms or may be present in CSF. One hundred and fifty-nine (159) microorganisms were spiked into negative CSF and assayed in triplicate. Some organisms with low concentrations (<1 x 10^6 CFU/mL for bacteria, fungi, cells or parasites; <1 x 10^5 IU/mL or PFU/mL or $TCID_{50}$/mL for viruses) were additionally tested *in silico*. For organisms not available to be tested, *in silico* analysis was performed. No cross-reactivity was observed. The results are presented in Table 5.

Table 5. Simplexa™ VZV Direct Cross-Reactivity (Analytical Specificity)

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
1	Adenovirus A12**	NA	NA
2	Adenovirus B35**	NA	NA
3	Adenovirus B7A	1 x 10^5 IU/mL	0.0% (0/3)
4	Adenovirus C1	1 x 10^5 IU/mL	0.0% (0/3)
5	Adenovirus C2	1 x 10^5 IU/mL	0.0% (0/3)
6	Adenovirus D20	1 x 10^5 IU/mL	0.0% (0/3)
7	Adenovirus E4**	NA	NA
8	Adenovirus F41**	NA	NA
9	*Aspergillus fumigatus*	1 x 10^6 CFU/mL	0.0% (0/3)
10	*Bacillus cereus*	1 x 10^6 CFU/mL	0.0% (0/3)
11	*Bacillus subtilis*	1 x 10^6 CFU/mL	0.0% (0/3)
12	BK virus	1 x 10^5 IU/mL	0.0% (0/3)
13	*Candida albicans*	1 x 10^6 CFU/mL	0.0% (0/3)

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
14	*Candida krusei*	1 x 10^6 CFU/mL	0.0% (0/3)
15	*Candida parapsilosis*	1 x 10^6 CFU/mL	0.0% (0/3)
16	*Candida tropicalis*	1 x 10^6 CFU/mL	0.0% (0/3)
17	*Citrobacter freundii*	1 x 10^6 CFU/mL	0.0% (0/3)
18	*Citrobacter koseri*	1 x 10^6 CFU/mL	0.0% (0/3)
19	Coronavirus 229E*	1 x 10^4 IU/mL*	0.0% (0/3)
20	Coronavirus NL63*	1 x 10^4 IU/mL*	0.0% (0/3)
21	Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
22	*Corynebacterium striatum***	NA	NA
23	*Corynebacterium urealyticum***	NA	NA
24	Coxsackievirus A10**	NA	NA
25	Coxsackievirus A16	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
26	Coxsackievirus A17**	NA	NA
27	Coxsackievirus A21*	1 x 10^4 $TCID_{50}$/mL*	0.0% (0/3)
28	Coxsackievirus A24**	NA	NA
29	Coxsackievirus A6**	NA	NA
30	Coxsackievirus A9	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
31	Coxsackievirus B1	1 x 10^5 IU/mL	0.0% (0/3)
32	Coxsackievirus B2	1 x 10^5 IU/mL	0.0% (0/3)
33	Coxsackievirus B3	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
34	Coxsackievirus B4	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
35	Coxsackievirus B5*	1 x 10^4 $TCID_{50}$/mL*	0.0% (0/3)
36	*Cronobacter sakazakii*	1 x 10^6 CFU/mL	0.0% (0/3)
37	*Cryptococcus albidus***	NA	NA
38	*Cryptococcus amylolentus***	NA	NA
39	*Cryptococcus gattii***	NA	NA
40	*Cryptococcus laurentii***	NA	NA

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
41	*Cryptococcus neoformans*	1 x 10^6 CFU/mL	0.0% (0/3)
42	*Cryptococcus uniguttulatus***	NA	NA
43	Cytomegalovirus (AD169 Strain)	1 x 10^5 IU/mL	0.0% (0/3)
44	Dengue virus (Type 1)*	1 x 10^4 IU/mL*	0.0% (0/3)
45	Dengue virus (Type 2)	1 x 10^5 IU/mL	0.0% (0/3)
46	Echovirus 1	1 x 10^5 IU/mL	0.0% (0/3)
47	Echovirus 11	1 x 10^5 IU/mL	0.0% (0/3)
48	Echovirus 18**	NA	NA
49	Echovirus 4	1 x 10^5 IU/mL	0.0% (0/3)
50	Echovirus 6	1 x 10^5 IU/mL	0.0% (0/3)
51	Echovirus 7	1 x 10^5 IU/mL	0.0% (0/3)
52	Echovirus 9	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
53	Encephalomyocarditis virus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
54	*Enterobacter aerogenes*	1 x 10^6 CFU/mL	0.0% (0/3)
55	*Enterobacter cloacae*	1 x 10^6 CFU/mL	0.0% (0/3)
56	Enterovirus 68**	NA	NA
57	Enterovirus 70	1 x 10^5 IU/mL	0.0% (0/3)
58	Enterovirus 71	1 x 10^5 IU/mL	0.0% (0/3)
59	Epstein-Barr virus (B95-8 Strain)	1 x 10^5 copies/mL	0.0% (0/3)
60	*Escherichia coli* (O157:H7)	1 x 10^6 CFU/mL	0.0% (0/3)
61	*Escherichia coli* K1**	NA	NA
62	*Escherichia fergusonii***	NA	NA
63	*Escherichia hermanii***	NA	NA
64	*Escherichia vulneris***	NA	NA
65	*Filobasidium capsuligenum***	NA	NA
66	*Haemophilus ducreyi*	1 x 10^6 CFU/mL	0.0% (0/3)
67	*Haemophilus haemolyticus***	NA	NA

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
68	*Haemophilus influenza* Type B	1 x 10^6 CFU/mL	0.0% (0/3)
69	*Haemophilus influenzae* Type A	1 x 10^6 CFU/mL	0.0% (0/3)
70	*Haemophilus parahaemolyticus***	NA	NA
71	*Haemophilus parainfluenzae*	1 x 10^6 CFU/mL	0.0% (0/3)
72	Hepatitis A virus	1 x 10^5 IU/mL	0.0% (0/3)
73	Hepatitis B virus	1 x 10^5 IU/mL	0.0% (0/3)
74	Hepatitis C virus	1 x 10^5 IU/mL	0.0% (0/3)
75	Hepatitis D virus**	NA	NA
76	HHV-6A	1 x 10^5 copies/mL	0.0% (0/3)
77	HHV-6B	1 x 10^5 copies/mL	0.0% (0/3)
78	HHV-7 SB	1 x 10^5 IU/mL	0.0% (0/3)
79	HHV-8	1 x 10^5 copies/mL	0.0% (0/3)
80	HIV-1 IIIB	1 x 10^5 IU/mL	0.0% (0/3)
81	HIV-2 NIHZ	1 x 10^5 IU/mL	0.0% (0/3)
82	HPeV-1**	NA	NA
83	HPeV-2**	NA	NA
84	HPeV-3	1 x 10^5 IU/mL	0.0% (0/3)
85	HPeV-4**	NA	NA
86	HPeV-5**	NA	NA
87	HPeV-6**	NA	NA
88	HSV-1 (MacIntyre)	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
89	HSV-2 (G)	1 x 10^5 IU/mL	0.0% (0/3)
90	Human Rhinovirus A16	1 x 10^5 IU/mL	0.0% (0/3)
91	Human Rhinovirus B3**	NA	NA
92	Human Rhinovirus B83**	NA	NA
93	Influenza A/California/7/2009	1 x 10^5 IU/mL	0.0% (0/3)
94	Influenza A/H1N1	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
95	Influenza B/Florida/02/2006	1 x 10^5 IU/mL	0.0% (0/3)
96	JC virus (MAD-4 strain)	1 x 10^5 IU/mL	0.0% (0/3)
97	*Klebsiella pneumoniae*	1 x 10^6 CFU/mL	0.0% (0/3)
98	La Crosse encephalitis virus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
99	*Listeria innocua***	NA	NA
100	*Listeria ivanovii***	NA	NA
101	*Listeria monocytogenes*	1 x 10^6 CFU/mL	0.0% (0/3)
102	Measles virus	1 x 10^5 IU/mL	0.0% (0/3)
103	*Morganella morganii***	NA	NA
104	Mumps virus	1 x 10^5 IU/mL	0.0% (0/3)
105	*Mycobacterium tuberculosis* genomic DNA	1.6 x 10^6 copies/mL	0.0% (0/3)
106	*Naegleria fowleri**	1.8 x 10^5 cells/mL*	0.0% (0/3)
107	*Neisseria gonorrhoeae*	1 x 10^6 CFU/mL	0.0% (0/3)
108	*Neisseria lactamica***	NA	NA
109	*Neisseria meningitidis* (serogroup A)	1 x 10^6 CFU/mL	0.0% (0/3)
110	*Neisseria meningitidis* (unencapsulated)**	NA	NA
111	*Neisseria mucosa*	1 x 10^6 CFU/mL	0.0% (0/3)
112	*Neisseria sicca***	NA	NA
113	*Pantoea agglomerans***	NA	NA
114	Parainfluenza Type 1	1 x 10^5 IU/mL	0.0% (0/3)
115	Parainfluenza Type 2	1 x 10^5 IU/mL	0.0% (0/3)
116	Parainfluenza Type 3	1 x 10^5 IU/mL	0.0% (0/3)
117	Parainfluenza Type 4	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
118	Parvovirus B19	1 x 10^5 IU/mL	0.0% (0/3)
119	Poliovirus (Type 3)	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
120	*Propionibacterium acnes*	1 x 10^6 CFU/mL	0.0% (0/3)
121	*Proteus mirabilis* Z050	1 x 10^6 CFU/mL	0.0% (0/3)

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
122	*Pseudomonas aeruginosa*	1 x 10^6 CFU/mL	0.0% (0/3)
123	Rabies virus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
124	Respiratory syncytial virus	1 x 10^5 IU/mL	0.0% (0/3)
125	Rhinovirus 1A	1 x 10^5 IU/mL	0.0% (0/3)
126	Rotavirus (Type Wa)*	1 x 10^4 IU/mL*	0.0% (0/3)
127	Rubella virus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
128	*Salmonella bongori***	NA	NA
129	*Salmonella enterica***	NA	NA
130	*Serratia marcescens*	1 x 10^6 CFU/mL	0.0% (0/3)
131	*Shigella boydii***	NA	NA
132	*Shigella flexneri*	1 x 10^6 CFU/mL	0.0% (0/3)
133	*Shigella sonnei***	NA	NA
134	Simian Virus type 40	1 x 10^5 IU/mL	0.0% (0/3)
135	St. Louis encephalitis virus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
136	*Staphylococcus aureus* (MRSA), COL	1 x 10^6 CFU/mL	0.0% (0/3)
137	*Staphylococcus capitis***	NA	NA
138	*Staphylococcus epidermidis* (MRSE)	1 x 10^6 CFU/mL	0.0% (0/3)
139	*Staphylococcus haemolyticus***	NA	NA
140	*Staphylococcus hominis***	NA	NA
141	*Staphylococcus lugdunensis***	NA	NA
142	*Staphylococcus saprophyticus*	1 x 10^6 CFU/mL	0.0% (0/3)
143	*Streptococcus agalactiae*	1 x 10^6 CFU/mL	0.0% (0/3)
144	*Streptococcus anginosus***	NA	NA
145	*Streptococcus bovis***	NA	NA
146	*Streptococcus dysgalactiae*	1 x 10^6 CFU/mL	0.0% (0/3)
147	*Streptococcus intermedius*	1 x 10^6 CFU/mL	0.0% (0/3)
148	*Streptococcus mutans*	1 x 10^6 CFU/mL	0.0% (0/3)

No.	Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
149	*Streptococcus oralis***	NA	NA
150	*Streptococcus pneumoniae*	1 x 10^6 CFU/mL	0.0% (0/3)
151	*Streptococcus pseudopneumoniae***	NA	NA
152	*Streptococcus pyogenes* Z018	1 x 10^6 CFU/mL	0.0% (0/3)
153	*Streptococcus salivarius*	1 x 10^6 CFU/mL	0.0% (0/3)
154	*Streptococcus sanguinis***	NA	NA
155	*Toxoplasma gondii*	1 x 10^6 tachyzoites/mL	0.0% (0/3)
156	*Treponema pallidum***	NA	NA
157	*Tropheryma whipplei***	NA	NA
158	West Nile virus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)
159	White Blood Cells (Human Genomic DNA)	1 x 10^6 cells/mL	0.0% (0/3)

* Additional testing was performed *in silico* due to low concentration of stock and also found not to be cross reactive with VZV.
** Tested *in silico* due to unavailability of the organism.

INTERFERENCE

The performance of the Simplexa™ VZV Direct assay was evaluated with potentially interfering substances that may be present in CSF samples at the concentrations indicated in the table below. A total of sixteen (16) potentially interfering substances were tested in a low positive VZV sample at approximately 2 times LoD (based on ≥ 95% detection rate) in CSF matrix and assayed in triplicate. No interference was observed at the interferent concentrations indicated. The results are presented in Table 6.

Table 6. Simplexa™ VZV Direct Interference

No.	Potential Interferent	VZV Strain	Interferent Concentration	Agreement with Expected Results (#Detected/#Total)
1	Acyclovir	Ellen	9.4 mg/mL	100.0% (3/3)
		9939	9.4 mg/mL	100.0% (3/3)
2	Albumin	Ellen	50 mg/mL	100.0% (3/3)
		9939	50 mg/mL	100.0% (3/3)
3	Bilirubin	Ellen	0.0125 mg/mL	100.0% (3/3)
		9939	0.0125 mg/mL	100.0% (3/3)
4	Casein	Ellen	9.0 mg/mL	100.0% (3/3)
		9939	9.0 mg/mL	100.0% (3/3)

No.	Potential Interferent	VZV Strain	Interferent Concentration	Agreement with Expected Results (#Detected/#Total)
5	Foscarnet	Ellen	0.6 mg/mL	100.0% (3/3)
		9939	0.6 mg/mL	100.0% (3/3)
6	Gamma globulin	Ellen	10.4 mg/mL	100.0% (3/3)
		9939	10.4 mg/mL	100.0% (3/3)
7	Glucose	Ellen	11 mg/mL	100.0% (3/3)
		9939	11 mg/mL	100.0% (3/3)
8	Hemoglobin	Ellen	3.5 mg/mL	100.0% (3/3)
		9939	3.5 mg/mL	100.0% (3/3)
9	Human Genomic DNA	Ellen	72 µg/mL	100.0% (3/3)
		9939	72 µg/mL	100.0% (3/3)
10	Immunoglobulin	Ellen	10 mg/mL	100.0% (3/3)
		9939	10 mg/mL	100.0% (3/3)
11	Lactate	Ellen	2.2 mg/mL	100.0% (3/3)
		9939	2.2 mg/mL	100.0% (3/3)
12	Topical Antiseptic	Ellen	5% (v/v)	100.0% (3/3)
		9939	5% (v/v)	100.0% (3/3)
13	Trans-Isolate Medium	Ellen	50% (v/v)	100.0% (3/3)
		9939	50% (v/v)	100.0% (3/3)
14	UTM	Ellen	50% (v/v)	100.0% (3/3)
		9939	50% (v/v)	100.0% (3/3)
15	White blood cells	Ellen	$2x10^7$ WBC/mL	100.0% (3/3)
		9939	$2x10^7$ WBC/mL	100.0% (3/3)
16	Whole Blood in EDTA	Ellen	10% (v/v)	100.0% (3/3)
		9939	10% (v/v)	100.0% (3/3)

INHIBITION BY OTHER MICROORGANISMS

The Simplexa™ VZV Direct assay was evaluated by testing the ability to identify VZV virus when other potentially inhibitory organisms are present. The panel of one hundred and sixty (160) potentially inhibitory organisms was individually spiked into a pool with a low concentration at approximately 2 times LoD (based on ≥ 95% detection rate) in CSF. No inhibition by other organisms was observed for VZV at the concentrations indicated in Table 7.

Table 7. Simplexa™ VZV Direct Interference

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
1	NA	Adenovirus A12**	NA	NA
2	NA	Adenovirus B35**	NA	NA
3	Ellen	Adenovirus B7A	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
4	Ellen	Adenovirus C1	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
5	Ellen	Adenovirus C2	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	88.9% (8/9)
6	Ellen	Adenovirus D20	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
7	NA	Adenovirus E4**	NA	NA
8	NA	Adenovirus F41**	NA	NA
9	Ellen	*Aspergillus fumigatus*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
10	Ellen	*Bacillus cereus*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
11	Ellen	*Bacillus subtilis*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
12	Ellen	BK virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
13	Ellen	*Candida albicans*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
14	Ellen	*Candida krusei*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
15	Ellen	*Candida parapsilosis*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
16	Ellen	*Candida tropicalis*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
17	Ellen	*Citrobacter freundii*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
18	Ellen	*Citrobacter koseri*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
19	Ellen	Coronavirus 229E	1 x 10^4 IU/mL*	100.0% (3/3)
	9939		1 x 10^4 IU/mL*	100.0% (3/3)
20	Ellen	Coronavirus NL63	1 x 10^4 IU/mL*	100.0% (3/3)
	9939		1 x 10^4 IU/mL*	100.0% (3/3)
21	Ellen	Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
22	NA	*Corynebacterium striatum***	NA	NA
23	NA	*Corynebacterium urealyticum***	NA	NA
24	NA	Coxsackievirus A10**	NA	NA
25	Ellen	Coxsackievirus A16	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
26	NA	Coxsackievirus A17**	NA	NA
27	Ellen	Coxsackievirus A21	1 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
	9939		1 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
28	NA	Coxsackievirus A24**	NA	NA
29	NA	Coxsackievirus A6**	NA	NA
30	Ellen	Coxsackievirus A9	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
31	Ellen	Coxsackievirus B1	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
32	Ellen	Coxsackievirus B2	1 x 10^5 IU/mL	100.0% (3/3)

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
33	Ellen	Coxsackievirus B3	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
34	Ellen	Coxsackievirus B4	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
35	Ellen	Coxsackievirus B5	1 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
	9939		1 x 10^4 $TCID_{50}$/mL*	100.0% (3/3)
36	Ellen	*Cronobacter sakazakii*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
37	NA	*Cryptococcus albidus***	NA	NA
38	NA	*Cryptococcus amylolentus***	NA	NA
39	NA	*Cryptococcus gattii***	NA	NA
40	NA	*Cryptococcus laurentii***	NA	NA
41	Ellen	*Cryptococcus neoformans*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
42	NA	*Cryptococcus uniguttulatus***	NA	NA
43	Ellen	Cytomegalovirus (AD169 Strain)	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
44	Ellen	Dengue virus (Type 1)	1 x 10^4 UI/mL*	100.0% (3/3)
	9939		1 x 10^4 IU/mL*	100.0% (3/3)
45	Ellen	Dengue virus (Type 2)	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
46	Ellen	Echovirus 1	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
47	Ellen	Echovirus 11	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
48	NA	Echovirus 18**	NA	NA

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
49	Ellen	Echovirus 4	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
50	Ellen	Echovirus 6	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
51	Ellen	Echovirus 7	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
52	Ellen	Echovirus 9	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
53	Ellen	Encephalomyocarditis virus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
54	Ellen	*Enterobacter aerogenes*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
55	Ellen	*Enterobacter cloacae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
56	NA	Enterovirus 68**	NA	NA
57	Ellen	Enterovirus 70	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
58	Ellen	Enterovirus 71	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
59	Ellen	Epstein-Barr virus (B95-8 Strain)	1 x 10^5 copies/mL	100.0% (3/3)
	9939		1 x 10^5 copies/mL	100.0% (3/3)
60	Ellen	*Escherichia coli* (O157:H7)	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
61	NA	*Escherichia coli* K1**	NA	NA
62	NA	*Escherichia fergusonii***	NA	NA
63	NA	*Escherichia hermanii***	NA	NA
64	NA	*Escherichia vulneris***	NA	NA
65	NA	*Filobasidium*	NA	NA

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
		*capsuligenum***		
66	Ellen	*Haemophilus ducreyi*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
67	NA	*Haemophilus haemolyticus***	NA	NA
68	Ellen	*Haemophilus influenzae* Type A	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
69	Ellen	*Haemophilus influenzae* Type B	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
70	NA	*Haemophilus parahaemolyticus***	NA	NA
71	Ellen	*Haemophilus parainfluenzae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
72	Ellen	Hepatitis A virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
73	Ellen	Hepatitis B virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
74	Ellen	Hepatitis C virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
75	NA	Hepatitis D virus**	NA	NA
76	Ellen	HHV-6A	1 x 10^5 copies/mL	100.0% (3/3)
	9939		1 x 10^5 copies/mL	100.0% (3/3)
77	Ellen	HHV-6B	1 x 10^5 copies/mL	100.0% (3/3)
	9939		1 x 10^5 copies/mL	100.0% (3/3)
78	Ellen	HHV-7 SB	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	88.9% (8/9)
79	Ellen	HHV-8	1 x 10^5 copies/mL	100.0% (3/3)
	9939		1 x 10^5 copies/mL	100.0% (3/3)
80	Ellen	HIV-1 IIIB	1 x 10^5 IU/mL	100.0% (3/3)

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
	9939		1 x 10^5 IU/mL	88.9% (8/9)
81	Ellen	HIV-2 NIHZ	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
82	NA	HPeV-1**	NA	NA
83	NA	HPeV-2**	NA	NA
84	Ellen	HPeV-3	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
85	NA	HPeV-4**	NA	NA
86	NA	HPeV-5**	NA	NA
87	NA	HPeV-6**	NA	NA
88	Ellen	HSV-1 (MacIntyre)	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
89	Ellen	HSV-2 (G)	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
90	Ellen	Human Rhinovirus A16	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
91	NA	Human Rhinovirus B3**	NA	NA
92	NA	Human Rhinovirus B83**	NA	NA
93	Ellen	Influenza A/California/7/2009	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
94	Ellen	Influenza A/H1N1	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
95	Ellen	Influenza B/Florida/02/2006	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
96	Ellen	JC virus (MAD-4 strain)	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
97	Ellen	*Klebsiella pneumoniae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
98	Ellen	La Crosse encephalitis virus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
99	NA	*Listeria innocua***	NA	NA
100	NA	*Listeria ivanovii***	NA	NA
101	Ellen	*Listeria monocytogenes*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
102	Ellen	Measles virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
103	NA	*Morganella morganii***	NA	NA
104	Ellen	Mumps virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
105	Ellen	*Mycobacterium tuberculosis* genomic DNA	1.6 x 10^6 copies/mL	100.0% (3/3)
	9939		1.6 x 10^6 copies/mL	100.0% (3/3)
106	Ellen	*Naegleria fowleri*	1.78 x 10^5 cells/mL*	100.0% (3/3)
	9939		1.78 x 10^5 cells/mL*	100.0% (3/3)
107	Ellen	*Neisseria gonorrhoeae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
108	NA	*Neisseria lactamica***	NA	NA
109	Ellen	*Neisseria meningitidis* (serogroup A)	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
110	NA	*Neisseria meningitidis* (unencapsulated)**	NA	NA
111	Ellen	*Neisseria mucosa*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
112	NA	*Neisseria sicca***	NA	NA
113	NA	*Pantoea agglomerans***	NA	NA
114	Ellen	Parainfluenza Type 1	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
115	Ellen	Parainfluenza Type 2	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
116	Ellen	Parainfluenza Type 3	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
117	Ellen	Parainfluenza Type 4	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
118	Ellen	Parvovirus B19	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
119	Ellen	Poliovirus (Type 3)	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
120	Ellen	*Propionibacterium acnes*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
121	Ellen	*Proteus mirabilis* Z050	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
122	Ellen	*Pseudomonas aeruginosa*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
123	Ellen	Rabies virus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
124	Ellen	Respiratory syncytial virus	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
125	Ellen	Rhinovirus 1A	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
126	Ellen	Rotavirus (Type Wa)	1 x 10^4 IU/mL*	100.0% (3/3)
	9939		1 x 10^4 IU/mL*	100.0% (3/3)
127	Ellen	Rubella virus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
128	NA	*Salmonella bongori***	NA	NA
129	NA	*Salmonella enterica***	NA	NA

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
130	Ellen	*Serratia marcescens*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
131	NA	*Shigella boydii***	NA	NA
132	Ellen	*Shigella flexneri*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
133	NA	*Shigella sonnei***	NA	NA
134	Ellen	Simian Virus type 40	1 x 10^5 IU/mL	100.0% (3/3)
	9939		1 x 10^5 IU/mL	100.0% (3/3)
135	Ellen	St. Louis encephalitis virus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
136	Ellen	*Staphylococcus aureus* (MRSA), COL	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
137	NA	*Staphylococcus capitis***	NA	NA
138	Ellen	*Staphylococcus epidermidis* (MRSE)	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
139	NA	*Staphylococcus haemolyticus***	NA	NA
140	NA	*Staphylococcus hominis***	NA	NA
141	NA	*Staphylococcus lugdunensis***	NA	NA
142	Ellen	*Staphylococcus saprophyticus*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
143	Ellen	*Streptococcus agalactiae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
144	NA	*Streptococcus anginosus***	NA	NA
145	NA	*Streptococcus bovis***	NA	NA
146	Ellen	*Streptococcus dysgalactiae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
147	Ellen	*Streptococcus*	1 x 10^6 CFU/mL	100.0% (3/3)

No.	VZV Strain	Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)
148	9939	*intermedius*	1 x 10^6 CFU/mL	100.0% (3/3)
149	Ellen	*Streptococcus mutans*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
150	NA	*Streptococcus oralis***	NA	NA
151	Ellen	*Streptococcus pneumoniae*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
152	NA	*Streptococcus pseudopneumoniae***	NA	NA
153	Ellen	*Streptococcus pyogenes* Z018	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
154	Ellen	*Streptococcus salivarius*	1 x 10^6 CFU/mL	100.0% (3/3)
	9939		1 x 10^6 CFU/mL	100.0% (3/3)
155	NA	*Streptococcus sanguinis***	NA	NA
156	Ellen	*Toxoplasma gondii*	1 x 10^6 Tachyzoites/mL	100.0% (3/3)
	9939		1 x 10^6 Tachyzoites/mL	100.0% (3/3)
157	NA	*Treponema pallidum***	NA	NA
158	NA	*Tropheryma whipplei***	NA	NA
159	Ellen	West Nile virus	1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
	9939		1 x 10^5 $TCID_{50}$/mL	100.0% (3/3)
160	Ellen	White blood cells (Human genomic DNA)	1 x 10^6 cells/mL	100.0% (3/3)
	9939		1 x 10^6 cells/mL	100.0% (3/3)

* Any organisms with low titer stock were analyzed *in silico* by BLAST (Basic Local Alignment Search Tool, NCBI, NIH).
** Tested *in silico* due to unavailability of the organism.

CARRY-OVER CONTAMINATION

An amplification carry-over for the Simplexa™ assays has been assessed. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was observed.

EXPECTED VALUES

The prevalence of VZV as determined by the Simplexa™ VZV Direct assay in a multi-site clinical study with prospectively collected specimens is shown in Table 8 below. The expected values for VZV per site varied between 0% and 7.4%. The overall prevalence for all sites was 2.2% (fourteen of six hundred thirty-seven (14/637).

Table 8. Prospective VZV prevalence as determined by Simplexa™ VZV Direct

Site ID	Total	Simplexa™ VZV Direct Positive Samples	VZV Prevalence
1	199	3	1.5%
3	181	2	1.1%
6	27	2	7.4%
7	71	0	0%
8	20	0	0%
10	139	7	5%
All	**637**	**14**	**2.2%**