510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY AND INSTRUMENT COMBINATION

A. 510(k) Number:

K190219

B. Purpose for Submission:

Clearance of device to detect and identify Varizella Zoster Virus DNA in cerebrospinal fluid

C. Measurand:

Varizella zoster virus DNA

D. Type of Test:

Realtime Polymerase Chain Reaction

E. Applicant:

DiaSorin

F. Proprietary and Established Names:

Simplexa VZV Direct, Simplexa VZV Positive Control Pack

G. Regulatory Information:

Table 1: Regulatory Information

Regulation Section	Classification	Product Code(s)	Panel
21 CFR 866.3970 - Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid	Class II	PLO	MI - Microbiology
21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	Class II	OOI	CH - Clinical Chemistry

H. Intended Use:

1. Intended use(s):
   SimplexaVZV Direct: 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. 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.

2. Indication(s) for use:
   Same as Intended Use

3. Special conditions for use statement(s):
   Rx - For Prescription Use Only
   For in vitro diagnostic use

4. Special instrument requirements:
   The Simplexa VZV Direct assay is for use with the LIAISON MDX instrument (with LIAISON MDX Studio Software)

I. 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, the Simplexa VZV Positive Control Pack, and the Direct Amplification Disc. The assay is performed on the LIAISON MDX instrument which is controlled by an external computer equipped with LIAISON MDX Studio Software.

The Direct Amplification Disk (DAD) consumable, disposable discs into which the PCR reagents and test samples are manually pipetted in, is compartmentalized into eight separate wedges and up to eight separate specimens or controls may be processed on each disc. The disc may be reused until all wedges have been utilized. Each wedge contains sample and reagent input wells, microfluidic channels and laser activated valves to control the fluid flow, and a reaction chamber.

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.

The LIAISON MDX instrument is a rapid real-time Polymerase Chain Reaction (PCR thermocycler used for the identification of nucleic acid from biological specimens. The LIAISON MDX instrument automates amplification, fluorescence-based detection and result interpretation. The LIAISON MDX system was previously referred to as the 3M Integrated Cycler system which was previously cleared via the premarket notification under K102314. Results are reported as "Detected", Not Detected", "invalid" (due to failure of the Internal Control or inadequate sample volume) or with a specific error code and may be printed.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   FilmArray Meningitis/Encephalitis (ME) Panel for use with FilmArray and FilmArray 2.0 systems

2. Predicate 510(k) number(s):
   K160462

3. Comparison with the predicate:

Table 2: General Device Characteristic Similarities

Similarities		
Item	**Predicate (K160462)**	**New Device (K190219)**
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	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 VZVinfection 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: The Simplexa VZV Positive Control Pack is intended to be used as a control with the

Similarities		
Item	**Predicate (K160462)**	**New Device (K190219)**
	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 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 central nervous system (CNS) infection. Not all agents of the central nervous system (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 central nervous system 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.	Simplexa VZV Direct kit. This control is not intended for use with other assays or systems.
Product Code	PLO	Same
Regulation Number	21 CFR 866.3970 – Device 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
General Technology	Fluorescence based PCR system	Same
Sample Type	CSF	Same
Automated System	Yes	Same

Table 3: General Device Characteristic Differences

Differences		
Item	**Predicate (K160462)**	**New Device (K190219)**
Number of Analytes	Multiplex	Singleplex
Additional Assay targets	Detection and identification from different bacteria, fungi and viruses causing signs and symptoms of meningitis and/or encephalitis	None
Extraction	Yes	None
Amplification	Nested PCR reactions	One PCR reaction
Detection	Melting Curve Analysis based on fluorescent double stranded DNA binding dye	Detection of fluorescence from fluorophore labeled probes
Instrument	FilmArray or FilmArray 2.0 system	LIAISON MDX

K. Standard/Guidance Document Referenced (if applicable):

- FDA Guidance: Format for Traditional and Abbreviated 510(k) - Guidance for Industry and FDA Staff, July 07, 2015
- FDA Guidance: Off-The-Shelf Software Use in Medical Devices: Guidance for Industry, FDA Reviewers, and Compliance, September 9, 1999
- FDA Guidance: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices: Guidance for Industry and FDA Staff, May 11, 2005
- FDA Guidance: General Principles of Software Validation: Guidance for Industry and FDA Staff, January 11, 2002
- FDA Guidance: Cybersecurity for Networked Medical Devices Containing Off-The-Shelf (OTS) Software: Guidance for Industry, January 14, 2005
- FDA Guidance: Guidance for Industry and Food and Drug Administration Staff: Post Market Management of Cybersecurity in Medical Devices (December 28, 2016)

L. Test Principle:

1. General Test Principle

   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 Direct Amplification Disc(DAD) and the LIAISON MDX (with LIAISON MDX Studio Software).

   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.

The Simplexa VZV Direct assay uses the DAD consumable. The DAD consumable is compartmentalized into eight separate wedges and up to eight separate specimens or controls may be processed on each disc. The disc may be reused until all wedges have been utilized.

The Simplexa VZV Direct assay is performed by manually pipetting PCR reagents and test samples into the DAD. Each wedge contains sample and reagent input wells, microfluidic channels and laser activated valves to control the fluid flow, and a reaction chamber. After loading the reagent and samples, the wells of the individual wedge are resealed with the original top foil and the tab is removed at the perforation. This disc is specifically designed to meter the amount of reagent (Reaction Mix) and sample that are placed into specific wells in the disc. After sealing the foil on each wedge, the DAD is loaded into the LIAISON MDX instrument and the run is initiated. The system uses a combination of centrifugal force and laser-controlled valves to combine metered aliquots of the unextracted samples and PCR reagents in the reaction/detection chamber of each wedge in the following manner:

Centrifugal force moves the fluid into the metering chambers. Excess reagent and sample are forced into the waste chambers by centrifugal force. At a specific point in the assay protocol the software triggers the laser to open the sample and reagent laser valves. This allows the fluid to pass from the sample and reagent metering wells into the reaction chamber.

After the centrifugal force has mixed sample and reagent within the reaction chamber, the amplification cycles begin. A sample is considered positive for a particular target if intensity of the optical reading crosses a particular threshold before a predetermined cut-off cycle.

2. Quality Control

   Two controls should be run with this test:

   a. Simplexa VZV Positive Control Pack may be used as an external control for Quality Control (QC) testing, training or proficiency testing. The Simplexa VZV Positive Control Pack comes with its own instruction for use.
   b. Negative/No Template Control (NTC) – not included in the kit

Table 4: Expected Results for Each Control

Control Type	VZV	DNA Internal Control (DNA IC)
SimplexaVZV Positive Control[a]	Detected	Not applicable[b]
No Template Control (NTC)	Not Detected	Valid

[a] Typical Ct values for the Positive Control range between 27-33.
[b] Detection of the Simplexa DNA Internal Control (DNA IC) is not required for a valid result when VZV is detected.

3. Interpretation Of Results

Upon completion of the run, the software automatically calculates and displays results. For each accession ID (Sample ID) entered, the software displays a result ("Detected", "Not Detected", "Invalid", "EC500, EC505 or EC515") for VZV.

a. **"Detected"** result indicates the presence of varicella-zoster virus DNA in the patient sample.

b. **"Not Detected"** result indicates the absence of varicella-zoster virus DNA in the patient sample.

c. **"Invalid"** result indicates inability to conclusively determine presence or absence of varicella-zoster virus DNA in the patient sample. This result may be due to 1) Internal Control (IC) failure, or 2) failure to detect sufficient specimen volume. The sample needs to be retested. See "Invalid Results" section below.

d. **"EC500"** Data processing error due to noise, weak or late amplification in the signal. Repeat the sample. If the problem persists, contact Technical Service.

e. **"EC505"** Insufficient information to determine whether amplification was present. If the problem persists, contact Technical Service.

f. **"EC515"** Internal control amplification is not within specification. Result is invalid, repeat the sample. If the problem persists, contact Technical Service.

The report can be printed and/or exported as needed.

Invalid Results: In case of an "Invalid" result, re-test the sample with a new Reaction Mix vial from the same kit or a new kit. If the problem is unresolved, contact DiaSorin Molecular Technical Services department.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:
The reproducibility of the Simplexa VZV Direct assay between laboratories was evaluated in a study performed by two operators at each of three sites over a period of five days using a panel of 4 contrived samples and positive and negative controls (see

below). On each day of testing each operator tested three replicates of each panel member (5 days x 3 sites x 2 operators x 3 replicates = 90 replicates per panel member). Table 5 below describes the reproducibility of all testing sites individually and combined and Table 6 below provides a quantitative summary of the reproducibility based on the CT values achieved for the reproducibility of the panel members. The following covariates were calculated: Between site, between day, between instrument, between run, and within run viability.

Each of the laboratories tested the following panel of six samples:

1. Simplexa VZV Direct Positive Control (PC)
2. Negative/No Template Control (NTC)
3. Four contrived sample pools prepared by spiking a specific concentration of the VZV strain into CSF with the following VZV strains and concentrations:
    - A low positive close to LoD for strain VZV 9939
    - A medium positive for strain VZV 9939
    - A low positive close to LoD for strain VZV Ellen
    - A medium positive for strain VZV Ellen.

Table 5: 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%	

Table 6: Quantitative Summary of Reproducibility

Analyte	Sample	N	Mean Ct	Inter Site		Inter Day		Inter Instrument		Inter Assay		Intra Assay		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
VZV (FAM)	9939 – LP	90	35.8	0.00	0.0	0.86	2.4	0.00	0.0	1.11	3.1	0.56	1.6	1.51	4.2
	9939 – MP	90	34.9	0.28	0.8	0.00	0.0	0.28	0.8	0.67	1.9	0.48	1.4	0.87	2.5
	Ellen – LP	90	36.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.79	2.1	0.79	2.1
	Ellen – MP	90	35.6	0.00	0.0	0.02	0.1	0.00	0.0	0.08	0.2	0.43	1.2	0.44	1.2
	PC	90	30.3	0.45	1.5	0.04	0.1	0.45	1.5	0.00	0.0	0.32	1.0	0.55	1.8
	NTC	90	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

Inter Lot Precision – Reaction Mix

The inter-lot variability of the Simplexa VZV Direct Reaction Mix was assessed with two instruments and three lots of reagents at Diasorin's internal site. The panel members were the same in concentration and formulation as the ones used in the reproducibility study above. Testing was performed across three non-consecutive days with 1 run for each instrument and lot per day (each by a different operator). The design: 2 instruments X 3 Lots X 2 runs/Day X 3 days = 36 replicates per panel member.

The inter lot precision results are shown in Table 7 (Qualitative Summary and CT values) and Table 8 (Agreement of results).

Table 7: Qualitative Summary of Reproducibility and VZV Ct Values by Lot Number

VZV (FAM Channel) - Summary of Qualitative Results & Ct Values:								
Panel Member	Lot- 1		Lot-2		Lot-3		All Lots Combined	
	% Detection (#Detected /#Tested)	(Replicates) Mean Ct ±SD (%CV)	% Detection (#Detected /#Tested)	(Replicates) Mean Ct ±SD (%CV)	% Detection (# Detected/ # Tested)	(Replicates) Mean Ct ±SD (%CV)	% Detection (# Detected /#Tested/)	(Replicates) Mean Ct ±SD (%CV)
Ellen-LP	100.0% (12/12)	(n= 12) 37.5 ± 0.38 (1.0%)	100.0% (12/12)	(n= 12) 37.3 ± 0.28 (0.8%)	100.0% (12/12)	(n= 12) 37.7 ± 0.85 (2.3%)	100.0% (36/36)	(n= 36) 37.5 ± 0.57 (1.5%)
Ellen-MP	100.0% (12/12)	(n= 12) 36.5 ± 0.44 (1.2%)	100.0% (12/12)	(n= 12) 36.4 ± 0.49 (1.4%)	100.0% (12/12)	(n= 12) 36.4 ± 0.31 (0.8%)	100.0% (36/36)	(n= 36) 36.4 ± 0.41 (1.1%)

9939-LP	100.0% (12/12)	(n= 12) 37.4 ± 0.96 (2.6%)	100.0% (12/12)	(n= 12) 37.7 ± 0.71 (1.9%)	100.0% (12/12)	(n= 12) 37.3 ± 0.49 (1.3%)	100.0% (36/36)	(n= 36) 37.5 ± 0.74 (2.0%)
9939-MP	100.0% (12/12)	(n= 12) 34.5 ± 0.85 (2.5%)	100.0% (12/12)	(n= 12) 34.5 ± 0.77 (2.2%)	100.0% (12/12)	(n= 12) 34.3 ± 0.98 (2.9%)	100.0% (36/36)	(n= 36) 34.5 ± 0.85 (2.5%)
CSF / Negative	0.0% (0/12)	(n= 12) N/A (N/A%)	0.0% (0/12)	(n= 12) N/A ± N/A (N/A%)	0.0% (0/12)	(n= 12) N/A ± N/A (N/A%)	0.0% (0/36)	(n= 36) N/A (N/A%)
Simplexa ™ VZV Direct Positive Control	100.0% (12/12)	(n= 12) 30.6 ± 0.42 (1.4%)	100.0% (12/12)	(n= 12) 30.4 ± 0.30 (1.0%)	100.0% (12/12)	(n= 12) 30.3 ± 0.35 (1.2%)	100.0% (36/36)	(n= 36) 30.4 ± 0.36 (1.2%)

Table 8: Agreement Reaction Mix Inter-lot Precision

Qualitative Summary of Reaction Mix Inter-lot Precision	
Panel Member	**Agreement % (# Correctly Identified/ # Tested) 95% CI**
Ellen – LP	100.0% (36/36) 95% CI: 90.4 to 100.0%
Ellen – MP	100.0% (36/36) 95% CI: 90.4 to 100.0%
9939 – LP	100.0% (36/36) 95% CI: 90.4 to 100.0%
9939 – MP	100.0% (36/36) 95% CI: 90.4 to 100.0%
NTC	100.0% (36/36) 95% CI: 90.4 to 100.0%
PC	100.0% (36/36) 95% CI: 90.4 to 100.0%

All panel members were correctly detected. The lot by lot variability with low and medium concentrations has a CV of less then 2.5%.

Inter-lot Precision- Simplexa Positive Control

The Inter-lot precision study was performed at DiaSorin's internal site. Three Simplexa VZV Direct Positive Control (PC) lots were used to evaluate inter-lot precision by performing a total of six runs across three non-consecutive days, two runs per day by a single operator using a single LIAISON MDX instrument and single lot of Simplexa VZV Direct Reaction Mix lot. Each run included at least two replicates per Simplexa VZV Direct Positive Control lot.The results of this study are summarized in Table 9 below.

Table 9: Summary of PC Inter-Lot Precision

Analyte	**N**	**Mean Ct**	**Inter-Lot**		**Inter-Day**		**Inter-Run**		**Intra-Run**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
VZV (FAM)	36	30.3	0.00	0.0	0.49	1.6	0.19	0.6	1.68	5.6	1.76	5.8
IC (Q670)	35*	29.2	0.02	0.1	0.06	0.2	0.00	0.0	0.14	0.5	0.15	0.5

* One IC Ct value was excluded from the statistical analysis of the internal control, since the IC is not required if valid amplification is observed in the FAM channel.

b. Linearity/assay reportable range:
Not applicable because the Assay is qualitative.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Stability study: The data support a shelf-life claim of 12 months for the Simplexa VZV Direct and the Simplexa VZV Positive Control Pack.

d. Cut-Off
Samples tested with the Simplexa VZV Direct assay are produce one of the following results Positive, Negative or Invalid based on the comparison of Cycle Threshold (Ct) values observed in the optical channels for VZV and the Internal Control targets to previously determined cut-offs. The Ct cut-offs were established during development of the assay and validated in the analytical and clinical study described in this Decision Summary.

e. *Detection Limit:*
The Limit of Detection (LoD) was determined for the Simplexa VZV Direct assay using quantified stocks of two VZV strains (Ellen and 9939) serially diluted into negative human CSF Dilutions were tested with a total of 32 replicates using 2 lots and 8 instruments across 4 non-consecutive days. The LoD was determined to be the lowest concentration that could be detected positive > 95% of the time (see Table 10 below).

Table 10 Summary of Limit of Detection

VZV Strain	Concentration ($TCID_{50}$/mL)	Logs copies/ mL
9939	2.03 $TCID_{50}$/mL	1.614
Ellen	0.001 $TCID_{50}$/mL	1.505

f. Analytical Reactivity:
The Simplexa VZV Direct assay was evaluated for analytical reactivity (inclusivity) to five (5) VZV strains (VZV 82, VZV 275, VZV 1700, VZV Isolate A and VZV Isolate B) in CSF. The study was performed with a single lot of Simplexa VZV Direct Reaction Mix and Simplexa VZV Direct Positive Control. The study was conducted over the course of two (2) non-consecutive days. Each titered strain was diluted into negative CSF at a concentration of 2.0 $TCID_{50}$/mL. Results are included in Table 11 below. An additional 178 strains of VZV were evaluated by *in silico* analysis (BLAST). All of these showed 100% homology, with the exception of Genbank KU52949. For Genbank KU52949, there was a 99% homology, with one mismatch in the Taqman probe region. This mismatch is not expected to negatively impact the detection of this strain.

Table 11: Analytical Reactivity of VZV Sstrains in CSF

Strain	#Detected/#Tested	Mean Ct (VZV)	SD
Strain 82	3/3	32.7	0.7
Strain 275	3/3	35.1	0.25
Strain 1700	3/3	38.0	0.26
Isolate A	3/3	31.7	0.12
Isolate B	3/3	33.9	0.32

g. Analytical Specificity:

Cross-Reactivity

The Simplexa VZV Direct assay was evaluated for cross reactivity by testing 105 different bacteria, viruses and fungi which represent closely related organisms or cause similar clinical symptoms. All samples were prepared by spiking each potentially cross reacting organism into VZV negative pooled human CSF. For potentially cross reacting bacteria or fungi, the testing concentration was $1x10^6$ CFU/mL; for viruses testing was performed at $1x10^5$ $TCID_{50}$/mL. No cross reactivity was observed for the tested organisms at the test concentration indicated in Table 12 below.

Competitive Interferences

The Simplexa VZV Direct was evaluated for microbial inhibition (or competitive interference by other microorganisms) by testing the same 105 different bacteria, viruses and fungi that were tested for cross reactivity.

All samples were prepared by spiking each potentially inhibiting organism into a baseline consisting of either the Ellen or 9939 strains of VZV at a concentration of 1-2x LoD in VZV- negative human CSF. For potentially inhibiting bacteria or fungi, the testing concentration was $1x10^6$ CFU/mL; for viruses the testing concentration was $1x10^5$ $TCID_{50}$/mL. For organisms not titered in CFU/mL or $TCID_{50}$/mL, other industry acceptable units were used. Results are included in the last column of Table 12 No microbial interference was observed for the tested organisms at the test concentration indicated in Table 12 below

Table 12: Simplexa VZV Direct Cross-Reactivity

Organism	Tested Concentration	% Detection of VZV	
		Without VZV (Cross Reactivity)	With VZV (Microbial Interference)
Adenovirus B7A	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Adenovirus C2	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Adenovirus D20	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Aspergillus fumigatus	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Bacillus cereus	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)

Organism	Tested Concentration	% Detection of VZV	
		Without VZV (Cross Reactivity)	With VZV (Microbial Interference)
Bacillus subtilis	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
BK virus	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Candida albicans	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Candida krusei	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Candida parapsilosis	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Candida tropicalis	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Citrobacter freundii	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Citrobacter koseri	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Coronavirus 229E	1 x 10^4 U/mL*	0.0% (0/3)	100% (3/3)
Coronavirus NL63	1 x 10^4 U/mL*	0.0% (0/3)	100% (3/3)
Coronavirus OC43	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus A16	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus A21	1 x 10^4 TCID50/mL*	0.0% (0/3)	100% (3/3)
Coxsackievirus A9	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus B1	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus B2	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus B3	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus B4	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Coxsackievirus B5*	1 x 10^4 TCID50/mL*	0.0% (0/3)	100% (3/3)
Cronobacter sakazakii	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Cryptococcus neoformans	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Cytomegalovirus (AD169 Strain)	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Dengue virus (Type1)	1 x 10^4 U/mL*	0.0% (0/3)	100% (3/3)
Dengue virus (Type2)	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Echovirus 1	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Echovirus 4	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Echovirus 6	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Echovirus 7	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Echovirus 9	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Echovirus 11	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Encephalo- myocarditis virus	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Enterobacter aerogenes	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Enterobacter cloacae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Enterovirus 70	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Enterovirus 71	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Epstein-Barr virus (B95-8 Strain)	1 x 10^5 copies/mL	0.0% (0/3)	100% (3/3)
Escherichia coli (O157:H7)	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Haemophilus ducreyi	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Haemophilus influenzae Type A	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Haemophilus influenza Type B	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Haemophilus parainfluenzae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)

Organism	Tested Concentration	% Detection of VZV	
		Without VZV (Cross Reactivity)	With VZV (Microbial Interference)
Hepatitis A virus	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Hepatitis B virus	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Hepatitis C virus	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
HHV-6A	1 x 10^5 copies/mL	0.0% (0/3)	100% (3/3)
HHV-6B	1 x 10^5 copies/mL	0.0% (0/3)	100% (3/3)
HHV-7 SB	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
HHV-8	1 x 10^5 copies/mL	0.0% (0/3)	100% (3/3)
HIV-1 IIIB	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
HIV-2 NIHZ	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
HPeV-3	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
HSV-1 (MacIntyre)	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
HSV-2 (G)	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Human Rhinovirus A16	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Influenza A/California/7/2009	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Influenza A/H1N1	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Influenza B/Florida/02/2006	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
JC virus (MAD-4 strain)	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Klebsiella pneumoniae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
La Crosse encephalitis virus	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Listeria monocytogenes	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Measles virus	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Mumps virus	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Mycobacterium tuberculosis genomic DNA	1.6 x 10^6 copies/mL	0.0% (0/3)	100% (3/3)
Naegleria fowleri	1.8 x 10^5 cells/mL*	0.0% (0/3)	100% (3/3)
Neisseria gonorrhoeae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Neisseria meningitides (serogroup A)	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Neisseria mucosa	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Parainfluenza Type 1	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Parainfluenza Type 2	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Parainfluenza Type 3	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Parainfluenza Type 4	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Parvovirus B19	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Poliovirus (Type 3)	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Propionibacterium acnes	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Proteus mirabilis Z050	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Rabies virus	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Respiratory syncytial virus	1 x 10^5 IU/mL	0.0% (0/3)	100% (3/3)
Rhinovirus 1A	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
Rotavirus (Type Wa)	1 x 10^4 U/mL*	0.0% (0/3)	100% (3/3)
Rubella virus	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Serratia marcescens	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)

Organism	Tested Concentration	% Detection of VZV	
		Without VZV (Cross Reactivity)	With VZV (Microbial Interference)
Shigella flexneri	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Simian Virus type 40	1 x 10^5 U/mL	0.0% (0/3)	100% (3/3)
St. Louis encephalitis virus	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
Staphylococcus aureus (MRSA), COL	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Staphylococcus epidermidis (MRSE)	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Staphylococcus saprophyticus	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus agalactiae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus dysgalactiae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus intermedius	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus mutans	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus pyogenes Z018	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	0.0% (0/3)	100% (3/3)
Toxoplasma gondii	1 x 10^6 tachyzoites/mL	0.0% (0/3)	100% (3/3)
West nile virus	1 x 10^5 TCID50/mL	0.0% (0/3)	100% (3/3)
White Blood Cells (Human Genomic DNA)	1 x 10^6 cells/mL	0.0% (0/3)	100% (3/3)

* Stocks for the organisms had insufficient titers. The organisms were additionally tested in silico and found to not be cross reactive.

Interfering substances

The Simplexa VZV Direct assay was evaluated for the effects of sixteen potentially interfering endogenous and exogenous substances.

All samples were prepared by spiking each potentially interfering substance into a baseline sample consisting of either of the two VZV strains (Ellen or 9939) at a level of 2x limit of detection (LoD), in pooled human CSF matrix. Each interferent was spiked into the baseline sample and tested in triplicate at the concentration listed in the Table 13 below.

Table 13: Simplexa VZV Direct Interference for the VZV ELLEN and 9939 strain combined

Potential Interferent	VZV Strain	Interferent Concentration	Agreement with Expected Results (#Detected/#Total)
Acyclovir	Ellen	9.4 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Albumin	Ellen	50 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Bilirubin	Ellen	0.0125 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Casein	Ellen	9.0 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)

Potential Interferent	VZV Strain	Interferent Concentration	Agreement with Expected Results (#Detected/#Total)
Foscarnet	Ellen	0.6 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Gammaglobulin	Ellen	10.4 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Glucose	Ellen	11 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Hemoglobin	Ellen	3.5 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Human Genomic DNA	Ellen	72 µg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Immunoglobulin	Ellen	10 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Lactate	Ellen	2.2 mg/mL	100.0% (3/3)
	9939		100.0% (3/3)
Topical Antiseptic	Ellen	5% (v/v)	100.0% (3/3)
	9939		100.0% (3/3)
Trans-Isolate Medium	Ellen	50% (v/v)	100.0% (3/3)
	9939		100.0% (3/3)
White blood cells	9939	$2x10^7$ WBC/mL	100.0% (3/3)
	ELLEN		100.0% (3/3)
Whole blood in EDTA	9939	10% (v/v)	100.0% (3/3)
	ELLEN		100.0% (3/3)

Carry -Over/Cross-contamination Study

A study to assess the potential for carry-over/cross-contamination was conducted in support of the Simplexa Flu A/B & RSV Direct assay (K120413). The study design was determined to be acceptable and no evidence of contamination was observed. Because of the similarities in format and assay workflow with this previously cleared device, no additional contamination studies were conducted with the Simplexa VZV Direct assay in support of this 510k.

Sample storage and handling - Fresh versus Frozen

The Sample Storage and Handling - Fresh Vs. Frozen Study was performed using contrived samples in CSF. For each strain (Ellen and 9939), 70 samples were contrived at various concentrations ranging from 2x LoD up to 50x LoD as indicated in the Table 14 (VZV Ellen strain) and Table 15 (VZV 9939 strain) below. Samples were tested fresh (Column "Fresh") and then transferred to refridgerated storage at 2-8 ºC for 72 hours. Subsequent to testing (Column "Refridgerated") samples were then transferred to the -70 ºC freezer where they were stored for 30 days. Samples were tested after a total of three freeze/Thaw cycles (Column "Frozen (including 3 Freeze Thaw Cycles)".

Table 14: Summary of Fresh vs. Frozen Stability for VZV Ellen Strain

Sample Concentration	Fresh	Refridgerated	3 Freeze Thaw Cycles
2x LoD	100.0% (40/40)	100.0% (40/40)	100.0% (40/40)
4x LoD	100.0% (15/15)	100.0% (15/15)	100.0% (15/15)
10x LoD	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
20x LoD	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
50x LoD	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
Combined	100.0% (70/70) 95% CI:94.9% - 100%	100.0% (70/70) 95% CI:94.9% - 100%	100.0% (70/70) 95% CI:94.9% - 100%

Table 15: Summary of Fresh vs. Frozen Stability for VZV 9939 Strain

Sample Concentration	Fresh	Refridgerated	3 Freeze Thaw Cycles
2x LoD	100.0% (40/40)	100.0% (40/40)	100.0% (40/40)
4x LoD	100.0% (15/15)	100.0% (15/15)	100.0% (15/15)
10x LoD	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
20x LoD	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
50x LoD	100.0% (5/5)	100.0% (5/5)	100.0% (5/5)
Combined	100.0% (70/70) 95% CI: 94.9% - 100%	100.0% (70/70) 95% CI: 94.9% - 100%	100.0% (70/70) 95% CI: 94.9% - 100%

Testing supports refridgerated sample storage at 2-8 for 72 hours and frozen sample storage at -70 ºC including up to 3 freeze thaw cycles.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable

   b. *Matrix comparison:*

      Not applicable

3. Clinical studies:

   a. *Clinical Sensitivity:* Not applicable

   b. *Clinical specificity:* Not applicable

   c. *Other clinical supportive data (when a. and b. are not applicable):*

Clinical Agreement:

A total of 724 remnant cerebrospinal fluid (CSF) samples from patients suspected of VZV infection of the central nervous system (CNS) were obtained from prospective and prospectively banked collections from eight collection sites during the Simplexa VZV Direct clinical study. Sites that prospectively collected samples (n= 10) performed testing of the samples directly and then shipped an aliquot of the sample to Diasorin Molecular for comparator testing. For the banked samples DiaSorin Molecular aliquoted and distributed the samples for Simplexa VZV Direct testing and the comparator testing.

Of the 724 total samples 637 clinical samples were included in the analysis based on the inclusion and exclusion criteria of the study. A total of 87 samples were excluded from the analysis for not having sufficient volume, for not generating a final comparator result, for not meeting the inclusion criteria, and for exceeding sample storage.

Simplexa VZV Direct results were compared to results from a composite reference method. The composite reference method consisted of two validated real-time PCR assays followed by confirmation of positive PCR amplification products with bi-directional sequencing. Samples were characterized as positive if one or both PCR assays were positive and confirmed by bi-directional sequencing. Samples were characterized as negative if both PCR assays were negative. All composite reference method testing was performed at DiaSorin Molecular. Table 16 below shows the Clinical Agreement of the Simplexa VZV Direct as compared to the composite reference method.

Table 16. Prospective Clinical Agreement Results for Simplexa VZV Direct vs. Composite Reference Method

Simplexa VZV Direct Results	Composite Reference Method (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 of two discordant results was positive with an alternate NAAT used by the 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 seven clinical sites in a blinded manner and 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%. The results of the Clinical Study stratified by the age of the subjects is shown in Table 17.

Table 17: Simplexa VZV Direct Clinical Study Results Stratified Patient Age and Gender

	Female			Male			Total		
Age	**N**	**Detected**	**Not Detected**	**N**	**Detected**	**Not Detected**	**N**	**Detected**	**Not Detected**
Birth to 1 month	5	0.0% (0/5)	100.0% (5/5)	6	0.0% (0/6)	100.0% (6/6)	11	0.0% (0/11)	100.0% (11/11)
>1 month to 2 years	6	0.0% (0/6)	100.0% (6/6)	11	0.0% (0/11)	100.0% (11/11)	17	0.0% (0/17)	100.0% (17/17)
>2 years to 12 years	8	0.0% (0/8)	100.0% (8/8)	6	0.0% (0/6)	100.0% (6/6)	14	0.0% (0/14)	100.0% (14/14)
>12 years to 21 years	21	0.0% (0/21)	100.0% (21/21)	13	7.7% (1/13)	92.3% (12/13)	34	2.9% (1/34)	97.1% (33/34)
>21 years to 60 years	191	1.6% (3/191)	98.4% (188/191)	155	3.2% (5/155)	96.8% (150/155)	346	2.3% (8/346)	97.7% (338/346)
>60 years	99	2.0% (2/99)	98.0% (97/99)	116	2.6% (3/116)	97.4% (113/116)	215	2.3% (5/215)	97.7% (210/215)
Total	330	1.5% (5/330)	98.5% (325/330)	307	2.9% (9/307)	97.1% (298/307)	637	2.2% (14/637)	97.8% (623/637)

4. Clinical cut-off**:** Not applicable

5. Expected values/Reference range: Not applicable

N. Instrument Name:

Liason MDX instrument

O. System Descriptions:

1. Modes of Operation:

Does the applicant’s device contain the ability to transmit data to a computer, webserver,

or mobile device?

Yes ____x____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ____x____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___x_____ or No _____

3. Specimen Identification:
   Specimens are identified by scanning a bar code label or by typing in an appropriate identifier.

4. Specimen Sampling and Handling:
   The Simplexa VZV Assay is for the use with CSF. Samples can be stored refrigerated for up to 72 hours, and can be stored freezed for up to 30 days.

5. Calibration:
   End-user calibration for for the LIASON MDX instrument is not necessary. Calibration of the optical modules (excitation and emission gain settings) is performed during the manufacturing process. And the values are stored in the instrument firmware.

6. Quality Control:
   The assay does not require calibration. The assay contains an internal control for PCR function. DiaSorin Molecular offers optional external QC materials that are intended for use with the assay. Controls are packaged in single use aliquots and stored frozen, once thawed the controls are stable for 30 minutes at laboratory temperature.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:
Not applicable.

Q. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.