Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K192376

B Applicant

DiaSorin Molecular LLC

C Proprietary and Established Names

Simplexa VZV Swab Direct, Simplexa VZV Positive Control Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PGI	Class II	21 CFR 866.3309 - Herpes Virus Nucleic Acid-Based Cutaneous And Mucocutaneous Lesion Panel	MI - Microbiology
PMN	Class II	21 CFR 866.3920 - Assayed quality control material for clinical microbiology assays	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination and FDA clearance for a new device.

B Measurand:

Varicella-zoster virus DNA

C Type of Test:

Realtime Polymerase Chain Reaction

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
Simplexa™ VZV Swab 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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

LIAISON MDX instrument

IV Device/System Characteristics:

A 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.

B Principle of Operation:

Nucleic acid amplification testing (NAAT)

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☐	☒
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name: LIAISON MDX
2. Specimen Identification: N/A
3. Specimen Sampling and Handling: N/A
4. Calibration: N/A
5. Quality Control: N/A

V Substantial Equivalence Information:

A Predicate Device Name(s):
Solana HSV 1+2/VZV Assay

B Predicate 510(k) Number(s):
K162451

C Comparison with Predicate(s):

Device & Predicate Device(s):	K192376	K162451
Device Trade Name	Simplexa VZV Swab Direct	Solana HSV 1+2/VZV Assay
General Device Characteristic Similarities		
Intended Use/ Indications for Use	The DiaSorin Molecular Simplexa VZV Swab Direct	The Solana HSV 1+2/VZV Assay is an *in vitro* diagnostic

	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.	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.
Automated System	Yes	Yes
Technology	Nucleic acid amplification	Same
General Device Characteristic Differences		
Instrument	LIAISON MDX	Solana instrument

VI Standards/Guidance Documents Referenced:

N/A

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/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. The combined results for all sites are presented in Table 1.

Table 1. 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%)

2. Linearity: N/A

3. Analytical Specificity/Interference:

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, 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 2.

Table 2. 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

Organism	Tested Concentration	Organism	Tested Concentration
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
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 potentially inhibitory organisms are present. A panel of ninety-nine (99) potential inhibitory organisms was individually spiked into pooled cutaneous and mucocutaneous swab matrix containing a low concentration of VZV at approximately 2X LoD and tested in triplicate. Table below references the microorganisms and their respective tested concentration. No inhibition was observed for the detection of either VZV Ellen or 9939 strain.

Table 3. 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

Organism	Tested Concentration	Organism	Tested Concentration
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
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 potential interfering endogenous and exogenous substances are indicated in the table below. 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 shown below.

Table 4. Simplexa VZV Swab Direct Interference

Potentially Interfering Substance.	VZV Strain	Active Ingredient	Tested Concentration	# Detected/ # Tested
Abreva	9939	Docosanol 10%	7% (w/v)	3/3
	Ellen			3/3
Acetaminophen	9939	N/A	7% (w/v)	3/3
	Ellen			3/3
Acyclovir	9939	N/A	10 mg/mL	3/3
	Ellen			3/3
Albumin	9939	N/A	10 mg/mL	3/3
	Ellen			3/3
Balneol lotion	9939	Buffers, emulsifiers, PEG, water, mineral oil, lanolin oil, preservatives	7% (v/v)	3/3
	Ellen			3/3
Carmex	9939	Camphor, 1.7%; Menthol, 0.7%	10% (w/v)	3/3
	Ellen			3/3
Casein	9939	N/A	10 mg/mL	3/3
	Ellen			3/3
Chlor-Trimeton	9939	Chlorpheniramine maleate	5 mg/mL	3/3
	Ellen			3/3
Cidofovir	9939	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Clotrimazole Vaginal Cream	9939	Clotrimazole	7% (w/v)	3/3
	Ellen		3.5% (w/v)	3/3
Cold-Eeze	Ellen	Zincum Gluconicum 2X	5% (w/v)	3/3
	9939		2.5% (w/v)	3/3
Cornstarch	9939	N/A	1.25 mg/mL	3/3
	Ellen			3/3
Denavir	9939	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Desitin	9939	Zinc Oxide, 40%	7% (w/v)	2/2
			3.5% (w/v)	3/3
	Ellen		3.5% (w/v)	3/3
Dextromethorphan hydrobromide (Robitussin-DM)	9939	Dextromethorphan hydrobromide	10 mg/mL	3/3
	Ellen			3/3
Douche	9939	N/A	7% (v/v)	3/3
	Ellen			3/3
Famciclovir	9939	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Feces	9939	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Foscarnet	9939	N/A	1.25 mg/mL	3/3
	Ellen			3/3
Glucose	9939	N/A	11 mg/mL	3/3
	Ellen			3/3
Gynol II contraceptive jelly	9939	Nonoxynol-9 (3%)	7% (w/v)	3/3
	Ellen			3/3
Human genomic DNA	9939	N/A	20 µg/mL	3/3

Potentially Interfering Substance.	VZV Strain	Active Ingredient	Tested Concentration	# Detected/ # Tested
	Ellen			3/3
Immunoglobulin	9939	N/A	10 mg/mL	3/3
	Ellen			3/3
KY Jelly	9939	N/A	10 mg/mL	3/3
	Ellen		5% (w/v)	3/3
Lactate	9939	N/A	2.2 mg/mL	3/3
	Ellen			3/3
Lanacane	9939	Benzethonium chloride, 0.2%; Benzocaine, 20%	7% (v/v)	3/3
	Ellen			3/3
Lip-Clear Lysine	9939	Zinc Oxide, 1.2%	7% (w/v)	3/3
	Ellen		3.5% (w/v)	3/3
Miconazole 1	9939	Miconazole nitrate, 26%	10% (w/v)	3/3
	Ellen			3/3
Miconazole 3	9939	Miconazole nitrate, 2%	10% (w/v)	3/3
	Ellen			3/3
Monistat 1 insert	9939	Miconazole nitrate, 1200 mg	7% (w/v)	3/3
	Ellen			3/3
Monistat 3 cream	9939	Miconazole nitrate 2%	7% (w/v)	3/3
	Ellen			3/3
Mouthwash (Listerine)	9939	Eucalyptol, 0.092%; Menthol, 0.042%; Methyl salicylate, 0.060%; Thymol, 0.064%	7% (v/v)	3/3
	Ellen			3/3
Mucin	9939	N/A	5% (w/v)	3/3
	Ellen			3/3
Preparation H	9939	N/A	10% (w/v)	3/3
	Ellen			3/3
Releev	9939	N/A	10% (w/v)	3/3
	Ellen			3/3
Seminal Fluid	9939	N/A	10% (v/v)	3/3
	Ellen			3/3
Tioconazole 1	9939	N/A	10% (w/v)	3/3
	Ellen			3/3
Toothpaste (Colgate)	9939	Sodium fluoride, 0.243%	7% (w/v)	3/3
	Ellen			3/3
Urine	9939	N/A	10% (v/v)	3/3
	Ellen			3/3
Vagisil creme	9939	Benzocaine (5%), Resorcinol (2%)	7% (w/v)	3/3
	Ellen			3/3
Valacyclovir	9939	N/A	2.5 mg/mL	3/3
	Ellen			3/3
Valgancyclovir	9939	N/A	2.5 mg/mL	3/3
	Ellen			3/3
White blood cells	9939	N/A	$5.5x10^7$ cells/mL	3/3
	Ellen			3/3
Whole Blood in EDTA	9939	N/A	10% (v/v)	3/3
	Ellen			3/3
YeastGard suppositories	9939	*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

4. Assay Reportable Range: N/A

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods): N/A

6. Detection Limit:

The limit of detection (LoD) was determined for the Simplex 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 UTM matrix. The LoD was determined to be the lowest concentration that could be detected positive ≥95% of the time. The LoD for each strain is presented in Table 5.

Table 5. Simplexa VZV Swab Direct Assay Limit of Detection

VZV Strain	LoD ($TCID_{50}$/mL)	LoD (Copies /mL)
993	0.77	800
Elle	0.054	3500

7. Assay Cut-Off: N/A

8. Accuracy (Instrument): N/A

9. Carry-Over:
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 study. 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 was performed to determine the potential for contamination when negative samples are tested 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.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Clinical Studies

The performance of the Simplexa VZV Swab Direct assay was established in a clinical study that included three 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 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 month to greater than 60 years of age. Of these specimens, 62.4% of the specimens were from female patients and 37.6% of the specimens were from male patients. Ten testing sites performed the Simplexa VZV Swab Direct assay on enrolled specimens and shipped the specimens to two comparator testing sites to test against a three 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 validated VZV polymerase chain reaction (PCR) assays followed by bi-directional sequencing. The comparator testing was performed by two sites. One testing site performed the VZV DSFA and/or culture isolation with DFA and another testing site conducted the two validated VZV PCR assays testing followed by bi-directional sequencing. The results of the study are presented in Table 6.

Table 6. Simplexa VZV Swab Direct Prospective Agreement Results

Prospective Study	**Composite Reference Method (CRM)**				**Total**	**Sensitivity 95% CI[c]**	**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.

[c] 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 out of three outcomes from one FDA Cleared NAAT PCR assay for VZV and two validated VZV PCR assays followed by bi-directional sequencing. The FDA Cleared NAAT was performed by one external site. DiaSorin Molecular performed the Simplexa VZV Swab Direct testing and different DiaSorin Molecular operators

performed the two 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 7.

Table 7. Simplexa VZV Swab Direct Retrospective Agreement Results

Retrospective Study	Composite Reference Method 2 (CRM 2)				Total	PPA 95% CI[b]	NPA 95% CI
	+		-				
	Simplexa VZV Swab Direct		Simplexa VZV Swab Direct				
	+	-	+	-			
Mucocutaneous	9	1[a]	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%

[a] 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.

[b] 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 out of three outcomes from one FDA Cleared NAAT assay and two 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 8.

Table 8. Simplexa VZV Swab Direct Contrived Agreement Results

Contrived Mucocutaneous Samples	Composite Reference Method 2 (CRM 2)				Total	PPA 95% CI[a]	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%

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

2. Matrix Comparison: N/A

C Clinical Studies:

1. Clinical Sensitivity: N/A

2. Clinical Specificity: N/A

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   N/A

D Clinical Cut-Off: N/A

E Expected Values/Reference Range:

In the prospective clinical study 452 cutaneous and mucocutaneous samples were tested by Simplexa VZV Swab Direct. The values for VZV detection per site varied between 0% and 75%. The overall expected value for all sites is 19.9%. The expected values per site are presented in Table 9 below.

Table 9. VZV Incidence as Determined by Simplexa VZV Swab Direct

Site ID	Total	# Detected	VZV Incidence % Detected
3	4	3	75.0%
5	61	24	39.9%
6	48	15	31.3%
7	38	13	34.2%
8	12	3	25.0%
9	108	1	0.9%
10	139	16	11.5%
11	28	15	53.6%
13	14	0	0.0%
Total	452	90	19.9%

F Other Supportive Instrument Performance Characteristics Data: N/A

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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