

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K151906

B. Purpose for Submission:

Clearance of New Device

C. Measurand:

Target DNA sequences from Herpes Simplex Virus type 1 (HSV 1) and Herpes Simplex Virus type 2 (HSV 2)

D. Type of Test:

An *in vitro* molecular diagnostic test for the direct, qualitative detection and differentiation of HSV 1 and HSV 2 DNA in cutaneous or mucocutaneous lesion specimens.

E. Applicant:

Luminex Corporation

F. Proprietary and Established Names:

ARIES[®] HSV 1&2 Assay

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3309
2. Classification: Class II
3. Product code: PGI
OOI
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

The ARIES[®] HSV 1&2 Assay is a real-time polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the direct detection and differentiation of Herpes Simplex Virus 1 and 2 (HSV 1 and HSV 2) DNA in cutaneous or mucocutaneous lesion specimens from symptomatic patients. The test is indicated for use as an aid in diagnosis of HSV infection in symptomatic patients. The ARIES[®] HSV 1&2 Assay is indicated for use on the ARIES[®] System.

Warning: The ARIES[®] HSV 1&2 Assay is not FDA cleared for use with cerebrospinal fluid (CSF). The assay is not intended to be used for prenatal screening.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

The ARIES[®] HSV 1&2 Assay uses the ARIES[®] System that is capable of automated nucleic acid extraction and purification from a clinical sample, real-time PCR detection and differentiation of nucleic acid sequences, and data analysis.

The ARIES[®] System instrument uses the ARIES Software v1.0. The ARIES[®] instrument software provides the interface between the ARIES Software v1.0 and the ARIES system hardware.

Note: Refer to K151917 for additional information regarding ARIES[®] System and related Software.

I. Device Description:

The ARIES[®] HSV 1 & 2 Assay is a polymerase chain reaction (PCR)-based qualitative in vitro diagnostic test for the direct detection and differentiation of herpes simplex virus (HSV) DNA from cutaneous and mucocutaneous lesion swab specimens.

The cutaneous and mucocutaneous lesion swab specimens are collected in Copan Universal Transport Medium and transported to the laboratory. The specimen is pipetted into a cassette specific to the ARIES[®] HSV 1&2 Assay. In the cassette, the specimen is lysed and nucleic acid is extracted using the HSV 1&2 Assay Kit and ARIES[®] System. An extractable sample processing control (SPC) target is present in the ARIES[®] HSV 1&2 assay cassette and is processed with the specimen. The Ct value of the SPC serves to verify proper specimen lysis and nucleic acid extraction, to identify PCR inhibition, if any, and verify proper function of the extraction system and real-time instrument. The Tm value of the SPC is used as a reference for determining the target Tm.

The extracted nucleic acid is transferred via magnetic beads to the ARIES[®] HSV 1 & 2 Kit lyophilized PCR reagents in the cassette that contain a primer pair specific to HSV 1 and HSV 2 and a primer pair specific to the SPC sequence. The specific primer pairs are labeled with distinct fluorophore labels. PCR amplification is performed and assay fluorescence is monitored on the ARIES[®] System. Incorporation of the quencher-labeled nucleotide causes a decrease in assay fluorescence. Following amplification, the reaction is slowly heated and fluorescence is monitored. The strands of the amplification products will separate at a specific melting temperature (Tm) that is determined by an increase in fluorescence as the strands are separated. The sequences between the PCR primer binding sites of the HSV 1 and HSV 2 amplicons have different base compositions that are distinguished by their different Tm values. The instrument

fluorescence output is analyzed and test results are determined using the ARIES[®] HSV 1 & 2 Kit assay protocol file. Total assay time, including extraction and PCR cycling, takes approximately two hours. A printed results report is generated.

The ARIES[®] HSV 1&2 Assay consists of two kits:

- ARIES[®] HSV 1&2 Assay Cassette Kit - contains 24 assay cassettes which contain the necessary reagents for sample extraction, nucleic acid purification and amplification.
- ARIES[®] HSV 1&2 Assay Protocol File Kit - contains the assay protocol file, package insert and Quick Guide which ship separately on a USB flash drive as part of the ARIES[®] HSV 1&2 Assay Protocol File Kit.

J. Substantial Equivalence Information:

1. Predicate device name(s):

illumigene[®] HSV 1&2 DNA Amplification Assay (Meridian Bioscience, Inc.)

Reference Method:

ELVIS[®] HSV ID/Typing Test System (Diagnostic Hybrid, Inc.) for clinical evaluation (K971662)

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

K151046

3. Comparison with predicate:

Similarities		
Device Characteristic	ARIES [®] HSV 1 & 2 Assay (New Device)	<i>illumigene</i> [®] HSV 1&2 DNA Amplification Assay (Predicate Device - K151046)
Intended use	<p>The ARIES[®] HSV 1&2 Assay is a real-time polymerase chain reaction (PCR) based qualitative <i>in vitro</i> diagnostic test for the direct detection and differentiation of Herpes Simplex Virus 1 and 2 (HSV 1 and HSV 2) DNA in cutaneous or mucocutaneous lesion specimens from symptomatic patients. The test is indicated for use as an aid in diagnosis of HSV infection in symptomatic patients. The ARIES[®] HSV 1&2 Assay is indicated for use on the ARIES[®] System.</p> <p>Warning: The ARIES[®] HSV 1&2 Assay is not FDA cleared for use with cerebrospinal fluid (CSF). The assay is not intended to be used for prenatal screening.</p>	<p>The <i>illumigene</i> HSV 1&2 DNA amplification assay, performed on the <i>illumipro-10</i>[™], is a qualitative <i>in vitro</i> diagnostic test for the direct detection and differentiation of herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) DNA in cutaneous and mucocutaneous lesion specimens from male and female patients suspected of Herpetic infections.</p> <p><i>illumigene</i> HSV 1&2 utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect HSV-1 and HSV-2 by targeting segments of the herpes simplex virus 1 and herpes simplex virus 2 genomes. Results from <i>illumigene</i> HSV 1&2 are used as an aid in the diagnosis of HSV infection in symptomatic patients.</p> <p>The assay is intended for use in hospital, reference or state laboratory settings. This device is not intended for nonlaboratory point-of-care use.</p> <p>Warning: <i>illumigene</i> HSV 1&2 is not FDA cleared for use with cerebrospinal fluid (CSF) or to aid in the diagnosis of HSV infections of the central nervous system (CNS). The device is not intended for prenatal screening.</p>
Specimen Types	Male and female cutaneous and mucocutaneous lesion swab specimens	Male and female cutaneous and mucocutaneous lesion swab specimens
Test Principle	DNA amplification	DNA amplification
Assay Results	Qualitative detection and differentiation of HSV-1 and HSV-2 DNA	Qualitative detection and differentiation of HSV-1 and HSV-2 DNA

Differences		
Device Characteristic	ARIES [®] HSV 1 & 2 Assay (New Device)	<i>illumigene</i> [®] HSV 1&2 DNA Amplification Assay (Predicate Device - K151046)
Sample extraction and Amplification Instrumentation	Automated sample extraction; Real-time PCR amplification/detection using the Luminex ARIES [®] System	Manual sample preparation; isothermal Loop Mediated Amplification (LAMP) using the <i>illumipro-10</i> [™] .
Detection Method	Pairs fluorescent-labeled primers with quencher labeled nucleotides. Measures decrease in assay fluorescence with each PCR cycle.	Visible Light Transmission (Turbidity).

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

L. Test Principle:

The ARIES[®] HSV 1&2 Assay chemistry is based on an expanded genetic alphabet technology, using the synthetic DNA base pair 2'-deoxy-5-methyl-isocytidine (iC): 2'-deoxyisoguanosine (iG). The isobases (iC and iG) pair specifically with each other and not with natural nucleotides. In addition, isobases are efficiently incorporated during PCR. During PCR amplification, a quencher-modified iGTP is incorporated by the polymerase opposite an iC and a fluorophore reporter attached to a PCR primer. If the target is present and is amplified, assay fluorescence decreases with every cycle as amplification product accumulates. The decrease in assay fluorescence is monitored in real time using the Luminex ARIES[®] Instrument. Following PCR, the amplification products are thermally denatured and assay fluorescence is monitored. The strands of the amplification products are separated and assay fluorescence increases, thus determining the melting temperature (T_m) profile of the amplicon. The sequences between the PCR primer binding sites of the HSV-1 and HSV-2 amplicons have different base compositions that are distinguished by their different melting temperatures using the ARIES[®] HSV 1&2 Assay Analysis Software.

The specimen is added to the sample chamber of an ARIES[®] HSV 1&2 Assay cassette. The cassette is then placed into an ARIES[®] System magazine. A magazine can hold up to six cassettes. The magazine is inserted into an ARIES[®] System, which can process two magazines simultaneously. A barcode on top of the HSV 1&2 Assay cassette is automatically scanned by the ARIES[®] System, associating a preloaded ARIES[®] HSV 1&2 Assay protocol file with the cassette. The HSV 1&2 Assay protocol file contains the necessary parameters to run the cassette, analyze data, and generate reports.

Once a run is started, the Sample Processing Control (SPC) is automatically added to the sample chamber of the cassette to control for sample lysis, recovery of extracted nucleic acid, detection of inhibitory substances, and confirmation of PCR reagent integrity. Sample and SPC lysis, as well as isolation and purification of nucleic acids, are automated within the ARIES[®] System and the ARIES[®] HSV 1&2 Assay cassette. Purified nucleic acids are automatically transferred to the

cassette’s PCR tube that contains the lyophilized HSV 1&2 Master Mix for the PCR amplification step. The HSV 1&2 Master Mix contains a primer pair specific to HSV 1 and HSV 2, and a second primer pair specific to the SPC sequence.

Interpretation of Sample Results

The ARIES® analysis software determines results for the sample and the sample processing control (SPC) based on the amplification cycle (Ct) value, the melting temperature (T_m) value, and T_m threshold values provided in the assay protocol file. All assay outcomes are described below.

Interpretation of Sample Results

Example	SPC		HSV			Call
	Ct Value	T _m Value	Ct Value	HSV 1 T _m Value	HSV 2 T _m Value	
1	N/A	+	+	+	+	HSV 1&2
2	N/A	+	+	+	-	HSV 1 Positive
3	N/A	+	+	-	+	HSV 2 Positive
4	+	+	-	-	-	HSV 1&2 Negative
5	+	+	>a	N/A	N/A	
6	-	+	>a / -	N/A	N/A	Invalid
7	N/A	-	N/A	N/A	N/A	
8	N/A	+	-	+	N/A	
9	N/A	+	-	N/A	+	

N/A: Not applicable. All possible outcomes will result in the same call.

^a Greater than the Ct cut-off value.

Invalid Results

In case of an “Invalid” result, re-test the sample with a new assay cassette. If the problem is unresolved, Luminex Technical Support should be contacted.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Reproducibility:*

The reproducibility of the ARIES® HSV 1&2 Assay was evaluated by testing one lot of ARIES® HSV 1&2 Assay Cassettes on two ARIES® instruments by two operators at each of three sites on five non-consecutive days.

The panel members were formulated with a single target present (HSV-1 MacIntyre strain or HSV-2 MS strain) at three concentrations: moderate positive (4 X LoD for both HSV 1 and HSV 2), low positive (1 X LoD for both HSV 1 and HSV 2), and high negative (0.1 X LoD for HSV 1 and 0.4 X LoD for HSV 2). A true negative sample, where no HSV was added, was also prepared using viral transport medium. Each panel

member was tested in replicates of three, for five days, at three study sites. Testing at each site was performed by two operators and each operator ran the panel once a day. The results from the reproducibility study for the ARIES[®] HSV 1&2 Assay are presented in the table below.

Reproducibility Panel Results

	Site 1				Site 2				Site 3				Total Agreement with Expected Results	95% Confidence Interval
	Agreement with Expected Results ^a	Avg T _m	% CV T _m	Avg T _m Deflection ^b	Agreement with Expected Results ^a	Avg T _m	% CV T _m	Avg T _m Deflection ^b	Agreement with Expected Results ^a	Avg T _m	% CV T _m	Avg T _m Deflection ^b		
HSV-1 Moderate Positive	30/30	85.5	0.16%	2.25E+06	30/30	85.5	0.12%	2.56E+06	30/30	85.6	0.18%	2.72E+06	90/90 (100%)	96.0-100%
HSV-1 Low Positive	30/30	85.5	0.16%	2.04E+06	30/30	85.6	0.16%	2.24E+06	30/30	85.5	0.16%	2.45E+06	90/90 (100%)	96.0-100%
HSV-1 High Negative	11/30	85.4	0.17%	1.39E+06	9/30	85.5	0.20%	2.33E+06	9/30	85.5	0.17%	2.06E+06	29/90 (32.2%)	22.8-42.9%
HSV-2 Moderate Positive	30/30	87.9	0.17%	2.17E+06	30/30	87.8	0.16%	2.52E+06	30/30	87.8	0.15%	2.43E+06	90/90 (100%)	96.0-100%
HSV-2 Low Positive	30/30	87.8	0.11%	1.95E+06	29/30	87.7	0.17%	2.23E+06	30/31	87.7	0.16%	2.04E+06	89/91 (97.8%)	92.3-99.7%
HSV-2 High Negative	30/30	87.7	0.19%	1.75E+06	30/30	87.7	0.14%	1.98E+06	23/30	87.7	0.15%	1.94E+06	83/90 (92.2%)	84.6-96.8%
HSV1&2 Negative	30/30	76.4	0.30%	2.76E+05	30/30	76.3	0.24%	3.11E+05	30/30	76.3	0.68%	3.34E+05	90/90 (100%)	96.0-100%

^a Agreement with expected results for the HSV 1&2 negative reflects SPC positivity since no HSV 1 or HSV 2 was detected. Expected result for HSV 1 Moderate Positive target was 100% HSV 1 Positive; for HSV 1 Low Positive was approximately 95% HSV 1 Positive; for HSV 1 High Negative was 20% to 80% HSV 1 Positive; for HSV 2 Moderate Positive target was 100% HSV 2 Positive; for HSV 2 Low Positive was approximately 95% HSV 1 Positive; for HSV 2 High Negative was 20% to 80% Positive; and for HSV 1&2 Negative was 100% HSV 1&2 Negative.

^b Average T_m deflection (RFU) was calculated using all of the positive replicates for that target type. Average T_m deflection for the HSV 1&2 Negative reflects SPC T_m deflection since no HSV 1 or HSV 2 was detected.

b. *Precision (Within-Laboratory Repeatability)*

The repeatability of the ARIES[®] HSV 1&2 Assay was evaluated using the same panel described in the reproducibility study above. The seven member panel was tested by two operators performing testing across multiple ARIES[®] instruments using one lot of ARIES[®] HSV 1&2 Assay Cassettes. Testing was performed for 10 days and included a total of 216 replicates used in assessing repeatability. Results of the repeatability study for the ARIES[®] HSV 1&2 Assay performed at one sites are presented in the table below.

Repeatability Panel Results^a

Target Type	Agreement with Expected Results ^b	95% Confidence Interval	Average T _m	% Coefficient of Variation T _m	Average T _m Deflection ^c
HSV 1 Moderate Positive	100% (72/72)	95.0 – 100%	85.6	0.17%	3.28E+06
HSV 1 Low Positive	100% (72/72)	95.0 – 100%	85.6	0.13%	2.88E+06
HSV 1 High Negative	45.80% (33/72)	34.0 – 58.0%	85.4	0.12%	2.18E+06
HSV 2 Moderate Positive	100% (72/72)	95.0 – 100%	87.9	0.16%	3.16E+06
HSV 2 Low Positive	100% (72/72)	95.0 – 100%	87.8	0.15%	2.75E+06
HSV 2 High Negative	97.40% (76/78)	91.0 – 99.7%	87.8	0.17%	2.39E+06
HSV 1&2 Negative	100% (72/72)	95.0 – 100%	76.5	0.66%	4.41E+05

^a An overall invalid rate of 0.8% (4/514) was observed.

^b Expected result for HSV 1 Moderate Positive target was 100% HSV 1 Positive; for HSV 1 Low Positive was approximately 95% HSV 1 Positive; for HSV 1 High Negative was 20% to 80% HSV 1 Positive; for HSV 2 Moderate Positive target was 100% HSV 2 Positive; for HSV 2 Low Positive was approximately 95% HSV 2 Positive; for HSV 2 High Negative was 20% to 80% HSV 2 Positive; and HSV 1&2 Negative was 100% HSV 1&2 Negative.

^c Average T_m deflection (RFU) was calculated using all of the positive replicates for that target type. Average T_m deflection for the HSV 1&2 Negative reflects SPC T_m deflection since no HSV 1 or HSV 2 was detected.

c. *Linearity/assay reportable range: N/A*

d. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Quality Control

Each ARIES® assay cassette contains a Sample Process Control (SPC), which is processed with the sample and analyzed during the amplification reaction. The SPC verifies sample lysis, nucleic acid extraction, and proper reagent, cassette, ARIES® System, and assay protocol performance. The SPC has a known melting temperature (T_m) range and Ct range. Each time an assay is run, the system measures the temperature and fluorescence intensity of the SPC control to ensure the thermal and optical subsystems have remained in calibration.

External Controls

External controls should be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. A reference HSV 1 and HSV 2 strain or well characterized HSV 1 and HSV 2 clinical isolates may be used as positive controls. Universal Viral Transport Medium may be used as a negative control. The Luminex ARIES® HSV 1&2 Assay Cassette Kit does not include external positive and negative control.

e. *Detection limit:*

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES® HSV 1&2 Assay using two representative strains of HSV 1 (MacIntyre and F) and two representative strains of HSV 2 (MS & G). Preliminary LoD concentrations were determined by performing a six-point, five-fold dilution series in Copan Universal Transport Media of each quantified (TCID₅₀/mL) strain. The observed LoD of a HSV strain was determined as the lowest concentration that had a positivity rate of ≥ 95%.

The LoD concentrations determined in the preliminary study were confirmed with the same HSV 1 and HSV 2 reference strains diluted to the preliminary LoD concentrations and tested with twenty-four (24) replicates. The final LoDs are presented in the table below.

Limit of Detection of the ARIES® HSV 1&2 Assay

HSV	Strain	LoD Concentration (TCID ₅₀ /mL)	Positivity
	MacIntyre	7.11E+03	24/24 (100%)
	F	16.5	23/24 (95.8%)
HSV 2	MS	2.7	24/24 (100%)
	G	2.8	24/24 (100%)

The final assay LoD claim is 7.11E+03 TCID₅₀/mL for HSV 1 (MacIntyre) and 2.7 TCID₅₀/mL for HSV 2 (MS). The analytical studies were performed using these two strains based on the final assay LoD claim.

f. Analytical specificity:

A study was performed to evaluate cross reactivity and interference of the ARIES[®] HSV 1&2 Assay with 61 microorganisms that might be found in cutaneous and mucocutaneous lesion specimens. The effect of potential cross reactivity or interference was evaluated by testing five replicates of each HSV 1, HSV 2 near the device's Limit of Detection (LoD), and negative replicates (Copan UTM) spiked with 61 potential cross reacting organisms. Bacteria were tested at 10⁶ cfu/ml or higher for bacteria and 10⁵ pfu/ml or higher for viruses. At the tested concentrations of the organisms, the organisms do not cross react or interfere with the assay. All HSV positive results were 100% positive and all HSV negative results were 100% negative.

Cross-Reacting and Microbial Interference Panel

Microorganism	Test Concentration
<i>Acinetobacter calcoaceticus</i>	9.27 x 10 ⁷ cfu/mL
<i>Bacteroides fragilis</i>	4.2 x 10 ⁸ cfu/mL
<i>Candida albicans</i>	1.74 x 10 ⁷ cfu/mL
<i>Candida glabrata</i>	7.87 x 10 ⁶ cfu/mL
<i>Chlamydia trachomatis</i>	1.8 x 10 ⁴ TCID ₅₀ /mL
<i>Clostridium sordellii</i>	4.9 x 10 ⁶ cfu/mL
Cytomegalovirus (AD169 Strain)	1.15 x 10 ⁶ TCID ₅₀ /mL
<i>Enterobacter cloacae</i>	7.43 x 10 ⁸ cfu/mL
<i>Enterococcus faecalis</i>	4.57 x 10 ⁸ cfu/mL
Enterovirus (Type 71)	4.17 x 10 ⁴ TCID ₅₀ /mL
Epstein-Barr virus (B95-8 Strain)	9.27 x 10 ⁷ copies/mL
<i>Escherichia coli</i>	5.13 x 10 ⁸ cfu/mL
<i>Gardnerella vaginalis</i>	5.43 x 10 ⁶ cfu/mL
Hepatitis A Virus	8.47 x 10 ² IU/mL
Hepatitis B Virus	5.62 x 10 ⁸ IU/mL
HIV-1	1.05 x 10 ⁵ TCID ₅₀ /mL

Microorganism	Test Concentration
Human Herpes 6 virus (Z29 Strain)	4.17 x 10 ⁴ TCID ₅₀ /mL
Human Herpes 7 virus (SB Strain)	1.15 x 10 ⁶ TCID ₅₀ /mL
Human Papilloma virus	1.68 x 10 ⁹ copies/mL
<i>Lactobacillus acidophilus</i>	2.00 x 10 ⁷ cfu/mL
<i>Legionella micdadei</i>	2.70 x 10 ⁸ cfu/mL
<i>Mobiluncus mulieris</i>	3.18 x 10 ⁸ cfu/mL
<i>Moraxella cartarrhalis</i>	9.90 x 10 ⁵ cfu/mL
<i>Mycoplasma hominis</i>	3.6 x 10 ⁶ cfu/mL
<i>Mycoplasma orale</i>	1.4 x 10 ⁸ cfu/mL
<i>Mycoplasma salivarium</i>	4.7 x 10 ⁶ cfu/mL
<i>Neisseria gonorrhoeae</i>	5.73 x 10 ⁷ cfu/mL
<i>Propionibacterium acnes</i>	3.7 x 10 ⁸ cfu/mL
<i>Proteus mirabilis</i>	2.10 x 10 ⁸ cfu /mL
Rubella virus	1.26 x 10 ⁵ TCID ₅₀ /mL
<i>Salmonella enteritidis</i>	2.08 x 10 ⁷ cfu/mL
<i>Serratia marcescens</i>	4.07 x 10 ⁸ cfu/mL
<i>Staphylococcus aureus</i>	1.42 x 10 ⁹ cfu/mL
<i>Staphylococcus epidermidis</i>	3.47 x 10 ⁸ cfu/mL
<i>Streptococcus pyogenes</i>	2.60 x 10 ⁸ cfu/mL
<i>Staphylococcus saprophyticus</i>	6.60 x 10 ⁶ cfu/mL
<i>Streptococcus agalactiae</i>	8.67 x 10 ⁷ cfu/mL
<i>Toxoplasma gondii</i>	6.6 x 10 ⁵ tachyzoites/mL
<i>Treponema pallidum</i>	9.8 x 10 ⁶ genome copies/mL
<i>Trichomonas vaginalis</i>	4.21 x 10 ⁵ trophozoites/mL
Varicella Zoster virus	2.45 x 10 ⁴ TCID ₅₀ /mL
<i>Acinetobacter Iwoffii</i>	8.27 x 10 ⁷ cfu/mL
<i>Haemophilus influenzae</i> type B	5.33 x 10 ⁷ cfu/mL
<i>Klebsiella pneumoniae</i>	6.28 x 10 ⁸ cfu/mL

Microorganism	Test Concentration
<i>Neisseria meningitides</i> Serogroup A	7.07 x 10 ⁸ cfu/mL
<i>Prevotella melaninogenica</i>	4.10 x 10 ⁶ cfu/mL
<i>Streptococcus mitis</i>	5.73 x 10 ⁷ cfu/mL
<i>Streptococcus mutans</i>	4.37 x 10 ⁸ cfu/mL
<i>Streptococcus pneumoniae</i>	9.2 x 10 ⁷ cfu/mL
<i>Streptococcus salivarius</i>	7.47 x 10 ⁷ cfu/mL
<i>Candida parapsilosis</i>	2.87 x 10 ⁶ cfu/mL
<i>Candida tropicalis</i>	2.15 x 10 ⁶ cfu/mL
Human genomic DNA	10 µg/mL
Adenovirus 2	5.01 x 10 ⁵ U/mL
<i>Candida guilliermondii</i>	1.78 x 10 ⁷ cfu/mL
<i>Candida krusei</i>	6.3 x 10 ⁶ cfu/mL
<i>Candida lusitaniae</i>	1.42 x 10 ⁸ cfu/mL
<i>Fusobacterium nucleatum</i>	N/A ^a
<i>Haemophilus ducreyi</i>	2.05 x 10 ⁶ cfu/mL
<i>Mobiluncus curtisii</i>	>10 ³ cfu/mL
Simian Virus type 40	2.8 x 10 ⁶ TCID ₅₀ /mL

^a Concentration information not available

g. Interfering Studies

This study was performed to evaluate potential interference with the ARIES[®] HSV 1&2 Assay with a panel of twenty-eight (28) potential interfering substances. All of the potentially interfering substances were tested at concentrations at or above physiological levels or typical usage levels with HSV 1 (MacIntyre) and HSV 2 (MS) strains. The study was carried out in the presence of HSV 1 and HSV 2 near the device's limit of detection (LoD) to evaluate potential interference with the detection of the HSV 1 and HSV 2 targets. Each potentially interfering substance was tested in 5 replicates of each HSV 1, HSV 2, and negative samples (Copan UTM). All HSV positive results were 100% positive and all negative results were 100% negative. No interference was observed with any of the substances tested.

Interfering Substance Panel

Interfering Substance	Test Concentration
Abreva (Docosanol)	10%
Acyclovir (Acycloguanosine)	2.5 mg/mL
Buffy Coat	5%
Carmex Cold Sore Lip Balm	1%
Casein	7.0 mg/mL
Clotrimazole 3 Vaginal Cream	1%
Toothpaste	5%
Anti-itch cream (Benzalkonium Chloride)	5%
Cidofovir	2.5 mg/mL
Douche	10%
Foscarnet	2.5 mg/mL
Ganciclovir	2.5 mg/mL
Valganciclovir	2.5 mg/mL
Leukocytes	10%
Lip Clear Lysine+	1%
Listerine	10%
Male Urine	10%
Female Urine	10%
Whole Blood	10%
Monistat 1	5%
Monistat 3	5%
Albumin	10 mg/mL
Releev Cold Sore Treatment	1%
K-Y Brand Jelly	5%
Spermicide	5%
Tioconazole	5%
Vagisil Cream	1%
YeastGard	1%

h. Specimen Stability in Universal Transport Media

Fresh Specimen Stability:

The performance of the ARIES® HSV 1&2 Assay was assessed for fresh specimen stability when stored at 2 – 8°C. The stability was assessed by testing 6 replicates of each of 7 HSV target concentrations across 6 different time points. The contrived specimens were prepared by spiking cultured organism into the negative clinical matrix (Copan Universal Transport Medium) at different levels. The concentrations tested were a moderate positive, low positive and high negative for HSV 1 and HSV 2 as well as a negative concentration. Moderate positive specimens gave the expected result of 100% positivity, low positive specimens gave the expected result of approximately 95% positivity (HSV 1: 97.2% positive and HSV 2 100% positive) and high negative specimens gave the expected result of 20 – 80% positivity (HSV 1: 25.0% positive and HSV 2: 63.8% positive). Negative specimens gave the expected result of 100% negativity. The data from this stability study support the claim in the package insert that the fresh specimens for the ARIES® HSV 1&2 Assay can be held at 2 - 8°C for up to 15 days.

Frozen Specimen Stability:

The objective of the frozen specimen stability was to evaluate the stability of specimens when stored at -65°C to -95°C. This was assessed by testing 6 replicates of each of 7 HSV contrived target concentrations in Copan Universal Transport Medium across 7 different time points extending out to 12 months. The concentrations used for testing were a moderate positive, low positive and high negative concentration for HSV 1 and HSV 2 as well as a negative specimen. The data up to 3 months has been collected with all targets yielding the expected result. Moderate positive specimens are 100% positive, low positive specimens are positive approximately 95% of the time, high negative specimens are positive 20 – 80% of the time and negative specimens are negative 100% of the time. The specimens are stable for up to 3 months when stored at -65°C to -95°C.

Note: The package insert claims that the specimens can be stored refrigerated at 2°C to 8°C for up to 15 days from the date of collection; if specimens will be used after 15 days from the date of collection, they should be stored frozen at $\leq -70^{\circ}\text{C}$.

i. Reagent Stability:

The objective of ARIES® HSV 1&2 Assay Cassette real time stability testing was to evaluate the stability of ARIES® HSV 1&2 Assay Cassette in order to establish a shelf life. This was assessed by testing 4 replicates of HSV 1, 4 replicates of HSV 2 and 4 replicates of negative (Copan UTM) targets on three different lots of ARIES® HSV 1&2 Assay Cassettes stored at 2 different temperatures (2 – 8°C and 25°C) at 10 different time points extending out to 19 months. Data up to 3 months has been collected and to date all targets for all lots and all storage temperatures have given the

expected result. HSV 1 replicates are 100% HSV 1 Positive, HSV 2 replicates are 100% HSV 2 Positive and negative replicates are 100% HSV 1&2 Negative. Therefore, ARIES® HSV 1&2 Assay Cassettes are stable for 3 months when stored at both 4°C and Room Temperature (25°C).

j. Co-infection

A study was designed to evaluate the ability of the ARIES® HSV 1&2 Assay to detect HSV-1 and HSV-2 analytes when both are present in one specimen. Analytes were tested at high (200X LoD) and low concentrations (5X LoD) using 12 replicates. The ARIES® HSV 1&2 Assay may not detect a co-infection of HSV 1 and HSV 2 in cases where the two virus types are not equally represented in clinical specimens. Co-infections were only detected when both analytes were present at 200X LoD. An HSV analyte at 5X LoD was not detected in the presence of a different HSV analyte at 200X LoD.

k. Carry-over/Cross Contamination

Carry-over and cross contamination for the ARIES® HSV 1&2 Assay was assessed by testing fifteen (15) high positive HSV 1 samples, 15 high positive HSV 2 samples and thirty (30) HSV negative samples (Copan UTM). Samples were tested in an alternating pattern with high positive samples run adjacent to negative samples across ten (10) consecutive runs. No carry-over and cross contamination was observed. The overall percent agreement was 100% for positive and negative samples.

l. Assay cut-off: Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The clinical performance evaluation was performed against a gold standard/reference method *i.e.*, Cell Culture using an enzyme linked virus inducible system with HSV typing by fluorescently labeled antibodies.

b. Matrix comparison: N/A

3. Clinical studies:

a. Clinical Sensitivity: N/A

b. Clinical Specificity: N/A

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

The performance of the ARIES® HSV 1&2 Assay was compared with the ELVIS® HSV ID/Typing Test System (Diagnostic Hybrid, Inc.) which is a gold

standard/reference method *i.e.*, Cell Culture using an enzyme linked virus inducible system with HSV typing by fluorescently labeled antibodies.

Clinical Performance

The clinical performance of the ARIES[®] HSV 1&2 Assay was assessed at three (3) geographically diverse clinical sites in the United States. A total of 1963 left-over clinical specimens from symptomatic male and female patients were included in the clinical study. Of these, 1500 specimens were prospectively collected (all comers). The remaining 463 were pre-selected for cutaneous and mucocutaneous lesion types that were under-represented in the initial prospective sample set. Additional oral and nasal lesions specimen lesion were also tested. All of the pre-selected specimens were also prospectively collected. Of the 1963 specimens tested, fifty-five (55) specimens were lesion sources from anatomical sites that could not be determined, four (4) specimens remained invalid upon re-testing by the ARIES[®] HSV 1&2 Assay and three were unavailable for re-testing. All of these 62 specimens were excluded from the clinical performance analysis.

The reference/comparative method used to evaluate the clinical performance of the ARIES[®] HSV 1&2 Assay was the ELVIS[®] HSV-ID and D³ Typing Test System. Because the ELVIS method provides no information on HSV 1 patient infected status (positive or negative) in specimens that test positive for HSV 2, all specimens that were positive for HSV 2 by the ELVIS[®] HSV-ID and D³ Typing System were excluded from the analysis of HSV 1 clinical performance.

A total of 448 cutaneous lesions specimens were tested. One hundred and one (101) specimens that were positive for HSV 2 by the ELVIS[®] HSV-ID and D³ Typing System were excluded from the analysis of HSV 1 clinical performance.

A total of 1453 mucocutaneous lesions specimens were tested. Two hundred and sixty three (263) specimens that were positive for HSV 2 by the ELVIS[®] HSV-ID and D³ Typing System were excluded from the analysis of HSV 1 clinical performance.

The performance of ARIES[®] HSV 1&2 Assay when compared to ELVIS[®] viral culture is summarized for cutaneous and mucocutaneous lesions in the tables below.

Summary of HSV 1 Results for Cutaneous Lesions (N=347)

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	Negative	TOTAL
Positive	51	17 ¹	68
Negative	5 ²	274	279
TOTAL	56	291	347
		95% CI	
Sensitivity	91.1% (51/56)	80.4% - 97.0%	
Specificity	94.2% (274/291)	90.8% - 96.6%	

¹Thirteen (13) HSV 1 ARIES positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. The remaining four (4) false positive specimens were negative for both HSV 1 and HSV 2 by bi-directional sequencing.

²All five (5) HSV 1 ARIES negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. One of these specimens was positive for HSV 2 by both ARIES and sequencing.

Summary of HSV 1 Results for Mucocutaneous Lesions (N=1190)

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	Negative	TOTAL
Positive	262	42 ¹	304
Negative	8 ²	878	886
TOTAL	270	920	1190
		95% CI	
Sensitivity	97.0% (262/270)	94.2% - 98.7%	
Specificity	95.4% (878/920)	93.9% - 96.7%	

¹Nineteen (19) HSV 1 ARIES® positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Twenty (20) false positive specimens were negative for both HSV-1 and HSV-2 by bi-directional sequencing. The remaining three (3) specimens were unavailable (QNS) for sequence analysis.

²Seven (7) HSV 1 ARIES® negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. One of these specimens was positive for HSV 2 by both ARIES® and sequencing. One (1) false negative specimen was positive for HSV-1 by bi-directional sequencing.

Summary of HSV 2 Results for Cutaneous Lesions (N=448)

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	Negative	TOTAL
Positive	96	39 ¹	135
Negative	5 ²	308	313
TOTAL	101	347	448
		95% CI	
Sensitivity	95.0% (96/101)	88.8% - 98.4%	
Specificity	88.8% (308/347)	85.0% - 91.9%	

¹Thirty five (35) HSV 2 ARIES positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. The remaining four (4) false positive specimens were negative for both HSV-1 and HSV-2 by bi-directional sequencing.

²All five (5) HSV 2 ARIES negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Two of these specimens were positive for HSV 1 by both ARIES and sequencing.

Summary of HSV 2 Results for Mucocutaneous Lesions (N=1453)

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	Negative	TOTAL
Positive	259	81 ¹	340
Negative	4 ²	1109	1113
TOTAL	263	1190	1453
		95% CI	
Sensitivity	98.5% (250/263)	96.2% - 99.6%	
Specificity	93.2% (1109/1190)	91.6% - 94.6%	

¹Fifty eight (58) HSV 2 ARIES positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Twenty-one (21) false positive specimens were negative for both HSV-1 and HSV-2 by bi-directional sequencing. The remaining two (2) specimens were unavailable (QNS) for sequence analysis.

²All four (4) HSV 2 ARIES negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Three (3) of these specimens were positive for HSV 1 by both ARIES and sequencing.

4. Clinical cut-off: N/A
5. Expected values/Reference range:

Prevalence: The prevalence of HSV-1 and HSV-2 with the ARIES® HSV 1&2 Assay was calculated for cutaneous and mucocutaneous specimens and is summarized for the combined sample set per age group and by specimen source in the table below.

Cutaneous Prevalence by Age

Age (years)	HSV 1			HSV 2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
0 - 10	10	4	40.0%	10	0	0.0%
11 - 20	53	16	30.2%	53	9	17.0%
21 - 30	125	19	15.2%	125	39	31.2%
31 - 40	85	17	20.0%	85	26	30.6%
41 - 50	63	5	7.9%	63	18	28.6%
51 - 60	50	4	8.0%	50	16	32.0%
>60	62	3	4.8%	62	27	43.5%
Not Determined	0	0	0.0%	0	0	0.0%
Overall	448	68	15.2%	448	135	30.1%

Cutaneous Prevalence by Lesion Source

Source	HSV 1			HSV 2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Genital - Penis	228	24	10.5%	22	74	32.5%
Skin Lesion	220	44	20.0%	22	61	27.7%
Overall	448	68	15.2%	44	135	30.1%

Mucocutaneous Prevalence by Age

Age (years)	HSV 1			HSV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
0 - 10	103	14	13.6%	103	3	2.9%
11 - 20	233	78	33.5%	233	47	20.2%
21 - 30	463	114	24.6%	463	127	27.4%
31 - 40	262	54	20.6%	262	62	23.7%
41 - 50	177	23	13.0%	177	48	27.1%
51 - 60	112	12	10.7%	112	26	23.2%
>60	95	8	8.4%	95	27	28.4%
Not Determined	8	1	0.0%	8	0	0.0%
Overall	1453	304	20.9%	1453	340	23.4%

Mucocutaneous Prevalence by Lesion Source

Source	HSV 1			HSV		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Anorectal	37	3	8.1%	37	14	37.8%
Genital Vaginal /Cervical	688	142	20.6%	688	187	27.2%
Genital Labia /Vulva	377	71	18.8%	377	121	32.1%
Urethral	25	4	16.0%	25	4	16.0%
Nasal	45	5	11.1%	45	5	11.1%
Ocular	43	5	11.6%	43	3	7.0%
Oral	238	74	31.1%	238	6	2.5%
Overall	1453	304	20.9%	1453	340	23.4%

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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