DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

MERIDIAN BIOSCIENCE, INC. September 9, 2015
STEFANIE JOHNS, PH.D.
REGULATORY AFFAIRS AND DESIGN ASSURANCE ASSOCIATE
3471 RIVER HILLS DRIVE
CINCINNATI, OH 45244

Re: K151046
Trade/Device Name: ***illumi****gene*® HSV 1&2 DNA Amplification Assay,
illumi*gene*® HSV 1&2 External Control Kit
Regulation Number: 21 CFR 866.3309
Regulation Name: Herpes virus nucleic acid-based cutaneous and mucocutaneous lesion panel
Regulatory Class: Class II
Product Code: PGI
Dated: April 16, 2015
Received: April 20, 2015

Dear Dr. Johns:

This letter corrects our substantially equivalent letter of July 17, 2015.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must

comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Stephen J. Lovell -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics and
Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement on last page.

510(k) Number *(if known)*

K151046

Device Name

***illumi**gene*® HSV 1&2 DNA Amplification Assay,
***illumi**gene*® HSV 1&2 External Control Kit

Indications for Use *(Describe)*

***illumi**gene*® HSV 1&2 DNA Amplification Assay:
The ***illumi**gene* HSV 1&2 DNA amplification assay, performed on the ***illumi**pro-10*™, is a qualitative in vitro 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.

***illumi**gene* 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 ***illumi**gene* HSV 1&2 are used as an aid in the diagnosis of HSV infection in symptomatic patients.

The assay is intended for use in hospital, reference or state laboratory settings. This device is not intended for nonlaboratory point-of-care use.

***WARNING: illumi**gene* 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.

***illumi**gene*® HSV 1&2 External Control Kit:
The ***illumi**gene* HSV 1&2 External Control Kit contains Positive and Negative Control Reagents for use with the ***illumi**gene* HSV 1&2 DNA Amplification Assay. External controls are used as part of a routine quality control program to aid the user in detection of unexpected conditions that may lead to test errors.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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510(k) Summary

510(k) number: **K151046** **Date of Preparation: 17 July 2015**

Owner:

Meridian Bioscience, Inc.
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Contact:

Primary Contact:
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Executive Vice President, Regulatory and Quality Systems

Trade Name: ***illumi****gene*® HSV 1&2 DNA Amplification Assay
illumi*gene*® HSV 1&2 External Controls

Common Name: Herpes simplex virus nucleic acid amplification assay

Classification Name: Herpes virus nucleic acid-based cutaneous and mucocutaneous lesion panel (21 CFR 866.3309, Product Code PGI)

Predicate Device: Quidel Corporation Lyra™ Direct HSV 1 + 2/VZV Assay
K133448

Device Description:

The ***illumi****gene* Molecular Diagnostic Test System is comprised of the ***illumi****gene*® HSV 1&2 DNA Amplification Assay Test Kit, the ***illumi****gene* HSV 1&2 External Controls Kit, and the ***illumi****pro-10*™ Automated Isothermal Amplification and Detection System.

The ***illumi****gene* HSV 1&2 molecular assay utilizes loop-mediated amplification (LAMP) technology to detect herpes simplex virus in cutaneous and mucocutaneous lesion swab specimens. Each ***illumi****gene* HSV 1&2 assay is completed using ***illumi****gene* Sample Preparation Apparatus III (SMP PREP III), ***illumi****gene* HSV 1 Test Devices, ***illumi****gene* HSV 2 Test Devices, Mineral Oil, ***illumi****gene* Heat Treatment Tubes, and transfer pipettes. Using a transfer pipette, specimens are added to SMP PREP III and dispensed into Heat Treatment Tubes. Samples are heat-treated to make target and control DNA available for amplification. Each heat-treated sample is added to one ***illumi****gene* HSV 1 and one ***illumi****gene* HSV 2 Test Device. Mineral oil is added to each ***illumi****gene* Test Device to prevent evaporation.

The ***illumi****pro-10*™ heats each ***illumi****gene* HSV 1 and HSV 2 Test Device containing prepared sample and control material, facilitating amplification of target DNA. When HSV-1 or HSV-2 is present in the specimen, a 208 base pair (bp) sequence of the HSV-1 glycoprotein G (US4) gene or a 189 bp sequence of the HSV-2 glycoprotein G (US4) gene is amplified and magnesium pyrophosphate is generated. Magnesium pyrophosphate forms a precipitate in the reaction mixture.

The ***illumi****pro-10*™ monitors the absorbance characteristics of the reaction solutions at the assay Run Start ($Signal_{initial}$, S_i) and at the assay Run End ($Signal_{final}$, S_f). The ***illumi****pro-10*™ calculates the change in light transmission between Run End and Run Start (S_f :S_i) and compares the ratio to an established cut-off value. The ***illumi****pro-10*™ performs this ratio calculation to both the TEST chamber and the CONTROL chamber.

Fixed cut-off values for the TEST chamber are used to report sample results. TEST chamber S_f:S_i ratios less than 82% are reported as 'POSITIVE'; TEST chamber S_f:S_i ratios greater than or equal to 82% are reported as 'NEGATIVE'. Numerical values are not reported.

Fixed cut-off values for the CONTROL chamber are used to determine validity. CONTROL chamber S_f:S_i ratios less than 90% are considered valid and allow for reporting of TEST chamber results (POSITIVE, NEGATIVE). CONTROL chamber S_f:S_i ratios greater than or equal to 90% are considered invalid and prevent reporting of TEST chamber results. Invalid CONTROL chamber reactions are reported as 'INVALID'. More stringent cut-off criteria are applied to the CONTROL chamber reaction to ensure amplification is not inhibited, reagents are performing as intended and that sample processing was performed appropriately.

The ***illumi****gene* HSV 1&2 External Controls Kit contains a combined HSV-1 and HSV-2 Positive Control and a Negative Control (Negative Control IV) for use in routine Quality Control testing. External Control reagents are provided to aid the user in detection of reagent deterioration, adverse environmental or test conditions, or variance in operator performance that may lead to test errors.

Intended Use:

The ***illumi****gene* HSV 1&2 DNA amplification assay, performed on the ***illumi****pro-10*™, is a qualitative in vitro 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.

illumi*gene* 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 ***illumi****gene* HSV 1&2 are used as an aid in the diagnosis of HSV infection in symptomatic patients.

The assay is intended for use in hospital, reference or state laboratory settings. This device is not intended for nonlaboratory point-of-care use.

WARNING: illumi*gene* 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.

Predicate Device Comparison:

Similarities		
	DEVICE ***illumi***gene® **HSV 1&2 DNA Amplification Assay**	**PREDICATE** **Quidel** **Lyra™ Direct HSV 1 + 2/VZV assay** **K133448**
Intended Use	Qualitative in vitro 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.	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.
Assay Results	Qualitative	Qualitative
Indications for Use	Professional Use	Professional Use
DNA Detected	Herpes simplex virus type 1 Herpes simplex virus type 2	Herpes simplex virus type 1 Herpes simplex virus type 2 Varicella-zoster virus
Typing of HSV-1 and HSV-2?	Yes	Yes
Specimen Types	Male and female cutaneous and mucocutaneous lesion swab specimens	Male and female cutaneous and mucocutaneous lesion swab specimens
Method	DNA Amplification	DNA Amplification
Detection	Instrument	Instrument

Differences		
	DEVICE ***illumi*gene® HSV 1&2 DNA Amplification Assay**	**PREDICATE** **Quidel** **Lyra™ Direct HSV 1 + 2/VZV assay** **K133448**
Amplification Methodology	Loop-Mediated Isothermal Amplification (LAMP)	Multiplex Real-Time PCR
Detection Methodology	Loop-Mediated Isothermal Amplification (LAMP)	Target-specific fluorescent-labeled hybridization probes.
Reagents/ Components	The ***illumi*gene** HSV 1&2 DNA Amplification Assay Kit contains ***illumi*gene** Sample Preparation Apparatus III, ***illumi*gene** HSV 1 Test Devices, ***illumi*gene** HSV 2 Test Devices, Mineral Oil, ***illumi*gene** Heat Treatment Tubes, and Transfer Pipettes.	The Lyra™ Direct HSV 1 + 2/VZV Assay kit consists of: - Rehydration Solution - Process Buffer Part M5050 (contains the PRC) - Lyra™ Direct HSV 1 + 2/VZV Master Mix Part M5012 - Lyophilized Contents: o DNA polymerase enzyme o Primers and probes o dNTPs o Stabilizers
Instrumentation	***illumi*pro-10™** Automated Isothermal Amplification and Detection System	Life Technologies QuantStudio™ Dx, the Applied Biosystems® 7500 Fast Dx, or the Cepheid SmartCycler® II System
Reading Method	Visible Light Transmission	Fluorescence detection of dual-labeled hydrolysis probes, Ct values.
Packaging	Supplied as a kit; 25 tests per kit	Supplied as a kit; 96 tests per kit.
Kit Storage	2-30°C	Reagents and Controls: 2-8°C
VZV detection?	No	Yes

NON-CLINICAL PERFORMANCE DATA

Analytical Performance:

***Precision/Reproducibility*:**
Reproducibility studies were carried out by three independent laboratories. Blind-coded panels of eighteen (18) samples were supplied to participating laboratories. Samples were randomly sorted within each panel to mask sample identities. Contrived HSV-1 (strain HF) and HSV-2 (strain MS) positive and negative samples were manufactured using simulated negative matrix (cheek swab matrix in MicroTest™ M4® viral transport medium). The panel included moderate positive samples (HSV-1: 2.16 x 10^4 $TCID_{50}$/mL or HSV-2: 4.80 x 10^3 $TCID_{50}$/mL), low positive samples (HSV-1: 1.08 x 10^4 $TCID_{50}$/mL or HSV-2: 2.40 x 10^3 $TCID_{50}$/mL), and two HSV-contrived negative samples (HSV-1 near cut-off: 308 $TCID_{50}$/mL, HSV-1 high negative: 29.7 $TCID_{50}$/mL; HSV-2 near cut-off:

24 $TCID_{50}$/mL, HSV-2 high negative: 2.2 $TCID_{50}$/mL). The panel also included two HSV-1 and HSV-2 negative samples and a Positive and Negative Control. Testing was performed by different operators at each site on the same day (intra-assay variability) for five days (inter-assay variability). Three lots of ***illumi****gene* HSV 1&2 and five ***illumi****pro-10* instruments were used in this study. Positive and Negative Controls were tested with each panel. Results are provided in the tables below:

Reproducibility Study Summary for HSV-1								
Sample Type	**Site 1**		**Site 2**		**Site 3**		**Total**	
	Percent Agreement		**Percent Agreement**		**Percent Agreement**		**Percent Agreement**	
HSV-1 Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
HSV-1 Low Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
HSV-1 & HSV-2 Near Cut-Off	20/30	66.7%	26/30	86.7%	20/30	66.7%	66/90	73.3%
HSV-1 & HSV-2 High Negative	29/30	96.7%	30/30	100.0%	29/30	96.7%	88/90	97.8%
HSV-1 Negative	60/60	100.0%	60/60	100.0%	60/60	100.0%	180/180	100.0%
Negative Control	10/10	100.0%	10/10	100.0%	10/10	100.0%	30/30	100.0%
Positive Control	10/10	100.0%	10/10	100.0%	10/10	100.0%	30/30	100.0%

Reproducibility Study Summary for HSV-2								
Sample Type	**Site 1**		**Site 2**		**Site 3**		**Total**	
	Percent Agreement		**Percent Agreement**		**Percent Agreement**		**Percent Agreement**	
HSV-2 Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
HSV-2 Low Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
HSV-1 & HSV-2 Near Cut-Off	25/30	83.3%	29/30	96.7%	25/30	83.3%	79/90	87.8%
HSV-1 & HSV-2 High Negative	29/30	96.7%	30/30	100.0%	29/30	96.7%	88/90	97.8%
HSV-2 Negative	60/60	100.0%	60/60	100.0%	60/60	100.0%	180/180	100.0%
Negative Control	10/10	100.0%	10/10	100.0%	10/10	100.0%	30/30	100.0%
Positive Control	10/10	100.0%	10/10	100.0%	10/10	100.0%	30/30	100.0%

***Detection Limit*:**
Analytical sensitivity studies were designed to determine the analytical limit of detection (LoD) of herpes simplex virus type 1 and type 2. The LoD is defined as the lowest concentration of analyte ($TCID_{50}$/mL) that can be distinguished from negative samples with a high degree of probability (95%). Two strains of HSV-1 (HF and MacIntyre) and two strains of HSV-2 (G and MS) diluted in a simulated negative cheek swab matrix were used to establish the LoD with three kit lots of ***illumi****gene* HSV 1&2. A minimum of four dilutions near the expected LoD were evaluated for each HSV strain during preliminary testing of twenty (20) individually prepared replicates. The preliminary LoD was confirmed through testing of an additional sixty (60) individually prepared replicates with HSV concentration at the LoD. The overall confirmed analytical LoD by HSV strain for ***illumi****gene* HSV 1&2 is summarized below:

HSV Type	Strain Description	LoD Concentration ($TCID_{50}$/mL)
Herpes simplex virus type 1	HF Strain (VR-260)	7.20×10^3
	MacIntyre Strain (VR-539)	9.89×10^4
Herpes simplex virus type 2	G Strain (VR-734)	1.20×10^3
	MS Strain (VR-540)	1.60×10^3

The final assigned LoD is 9.89×10^4 $TCID_{50}$/mL for HSV-1 and 1.60×10^3 $TCID_{50}$/mL for HSV-2.

Analytical sensitivity of ***illumi****gene* HSV 1&2 was verified through testing of twenty (20) HSV-1 and twenty (20) HSV-2 confirmed positive clinical samples from a variety of anatomical locations (i.e. oral, genital, anorectal, etc.) at the assay LoD. Each clinical sample was quantified and diluted to 9.89×10^4 $TCID_{50}$/mL or 1.60×10^3 $TCID_{50}$/mL for HSV-1 and HSV-2, respectively; samples quantified at concentrations less than the limit of detection were tested undiluted. All samples were detected by ***illumi****gene* HSV 1&2 at LoD or lower, except one HSV-1 clinical sample, which was detected at 7.20×10^7 $TCID_{50}$/mL.

Analytical Specificity:

***Interference Testing*:**
Non-microbial contaminants potentially found in cutaneous and mucocutaneous swab specimens were evaluated for the potential to interfere with the ***illumi****gene* HSV 1&2 assay. Each potentially interfering substance was added to contrived HSV-1 (strains HF and MacIntyre) and HSV-2 (strains G and MS) positive and negative samples at final concentrations of 60 µg/mL, 7% v/v, 7% w/v, or greater.

No interference was observed with the following substances:
(1) At concentrations of 7% w/v or v/v: Abreva® (10% Docosanol), Balneol® Hygienic Cleansing Lotion, Carmex® Original Lip Balm (1.7% camphor, 0.7% menthol), Desitin® (40% zinc oxide), Douche (CVS Pharmacy® Disposable), Fluoride toothpaste (Crest®, 0.243% sodium fluoride), K-Y® Brand Jelly, Lanacane® (0.2% benzethonium chloride, 20% benzocaine), Lip Clear® Lysine+® (1.2% zinc oxide), Miconazole 3 (CVS™ Yeast Infection Relief: 2% miconazole nitrate), Mouthwash (Listerine® Original: 0.092% eucalyptol, 0.042% menthol, 0.060% methyl salicylate, 0.064% thymol), Preparation H® Hemorrhoidal Ointment (14% mineral oil, 74.9% petrolatum, 0.25% phenylephrine HCl), Releev® (0.13% benzalkonium chloride), Tioconazole, Vagisil® Regular Strength (5% benzocaine, 2% resorcinol), Yeast Guard® Gel Treatment (Candida albicans, 27X HPUS; Candida parapsilosis, 27X HPUS; Pulsatilla, 27X HPUS), Feces, Seminal fluid, Urine, Whole blood, Buffy Coat.
(2) At concentrations of: 60 µg/mL – Mucus (Mucin, bovine submaxillary gland type I-S); 1.25 mg/mL – Cornstarch; 3.3 mg/mL – Albumin; 5 mg/mL – Acetaminophen, Casein, Chlorpheniramine maleate; 7 mg/mL – Acyclovir; 10 mg/mL – Acetylsalicylic acid, Dextromethorphan hydrobromide.

Casein at concentrations greater than 5 mg/mL was found to interfere with the assay.

Cold-EEZE® Cold Remedy plus Sore Throat (zincum gluconicum 2X) was tested at 7% v/v and produced invalid results in all replicates.

***Cross-Reactivity Study*:**
Crossreactivity studies employed contrived positive (HSV-1 strain HF and HSV-2 strain MS) and negative specimens inoculated with bacterial or fungal organisms to a minimum concentration of 1.0 x 10^6 CFU/mL (1.0 x 10^6 copies/mL where genomic DNA was used) or virus at a minimum of 1.0 x 10^5 $TCID_{50}$/mL (1.0 x 10^6 copies/mL where genomic DNA or RNA was used). None of the following organisms or their genetic material reacted with ***illumi****gene* HSV 1&2:

Acinetobacter calcoaceticus, Acinetobacter lwoffii, Bacteroides fragilis, Bordetella bronchiseptica, Bordetella pertussis, Candida albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chlamydia trachomatis, Chlamydophila pneumoniae, Clostridium difficile, Clostridium perfringens, Corynebacterium diphtheriae, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli (ESBL)*, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus ducreyi, Haemophilus influenzae* (Type A)*, Klebsiella pneumoniae, Lactobacillus acidophilus, Legionella pneumophila, Mobiluncus curtisii, Mobiluncus mulieris, Moraxella catarrhalis, Mycoplasma hominis, Mycoplasma orale, Mycoplasma pneumoniae, Mycoplasma salivarium, Neisseria gonorrhoeae, Neisseria meningitidis, Prevotella melaninogenica, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus mitis, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius, Toxoplasma gondii, Treponema palladium, Trichomonas vaginalis, Ureaplasma urealyticum*, Adenovirus, Coronavirus, Coxsackievirus, Cytomegalovirus, Echovirus, Enterovirus, Epstein Barr virus, Influenza A virus, Influenza B virus, Hepatitis B virus, Hepatitis C virus, Human herpes 6 virus, Human herpes 7 virus, Human herpes 8 virus, Human immunodeficiency virus type 1, Human metapneumovirus, Human papilloma virus, Measles virus, Mumps virus, Parainfluenza virus, Respiratory syncytial virus, Rubella virus, Varicella zoster virus.

Human genomic DNA was nonreactive at 1.0 x 10^6 copies/mL. In addition, there was no competitive inhibition observed from the organisms listed above with HSV-1 or HSV-2 in the ***illumi****gene* HSV 1&2 assay.

***Assay cut-off*:**
The ***illumi****gene* HSV 1&2 assay is manufactured with fixed cut-off values. The product is designed with a pre-selected cut-off value and amplification reagent concentrations are optimized to ensure appropriate reactions are obtained. Development optimization includes evaluation of characterized positive and negative clinical specimens. Amplification reagent concentrations are adjusted during design as needed to ensure ***illumi****gene* results are aligned with clinical specimen reported results.

Cut-off values applied in the following manner:

The ***illumi****pro-10*™ calculates the ratio of the Run End (Signal final or S_f) reads with the Run Start (Signal initial or S_i) reads and compares the ratio to an established cut-off value. The ***illumi****pro-10* performs this ratio calculation to both the TEST chamber and the CONTROL chamber.

Fixed cut-off values for the CONTROL chamber are used to determine validity. CONTROL chamber S_f:S_i ratios less than 90% are considered valid and allow for reporting of TEST chamber results (POSITIVE, NEGATIVE). CONTROL chamber S_f:S_i ratios greater than or equal to 90% are considered invalid. Results are reported as 'INVALID'; Test chamber results are not reported. More stringent cut-off criteria are applied to the Control chamber reaction to ensure amplification is not inhibited, reagents are performing as intended and that sample processing was performed appropriately.

Fixed cut-off values for the TEST chamber are used to report sample results. TEST chamber S_f:S_i ratios less than 82% are reported as 'POSITIVE'; TEST chamber S_f:S_i ratios greater than or equal to 82% are reported as 'NEGATIVE'. Numerical values are not reported.

CLINICAL PERFORMANCE DATA

Clinical Studies:

Clinical trials for the ***illumigene***® HSV 1&2 DNA Amplification Assay, including the ***illumipro-10***™ Automated Isothermal Amplification and Detection System, were conducted between October 2014 and March 2015. Performance characteristics of the assay were established with cutaneous and mucocutaneous lesion swab specimens from patients suspected of herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) infection. Performance characteristics of the ***illumigene*** HSV 1&2 assay were established by comparison to the ELVIS® HSV ID and D^3 Typing Test System.

A total of 1158 leftover, de-identified lesion swab specimens from symptomatic male and female patients were evaluated. One sample was HSV positive by ELVIS, however, could not be identified as HSV-1 or HSV-2 (indeterminate) and another sample was tested with contaminated ELVIS cells and could not produce a result (indeterminate). Both samples were excluded from the performance analysis. . One invalid result for HSV-1 and one invalid result for HSV-2, which could not be repeated by the ***illumigene***® HSV 1&2 DNA Amplification Assay, were also excluded from only the HSV-1 and the HSV-2 performance analysis, respectively. In addition, the ELVIS HSV ID and D^3 Typing Test System is unable to detect HSV-1 if HSV-2 has been identified first in co-infected specimens. Therefore, if a specimen was HSV-2 positive by ELVIS it was removed from the calculation of the HSV-1 performance analysis. One hundred and eighty one (181) specimens were identified as HSV-2 positive by ELVIS and were removed from the HSV-1 data analysis, including two specimens identified as ***illumigene*** positive for both HSV-1 and HSV-2. The swab specimens were categorized as cutaneous (skin lesion, genital – penis) or mucocutaneous (anorectal, genital-vaginal/cervical, nasal, ocular, oral, and urethral). The tables below include performance of the remaining 974 specimens tested for HSV-1 and 1155 specimens tested for HSV-2 for all sites combined.

Combined Sites: HSV-1 Cutaneous (N=264)

		Reference Method			*illumigene*	Performance			
		Pos	**Neg**	**Total**	**INV[c]**				**95% CI**
***illumigene* HSV 1&2**	**Pos**	48	6[a]	54	0 (0)	Sensitivity	48/51	94.1%	84.1-98.0%
	Neg	3[b]	207	210	0 (0)	Specificity	207/213	97.2%	94.0-98.7%
	Total	51	213	264	0 (0)				

[a] 6/6 samples identified as HSV-1 positive by an alternative, FDA-cleared molecular assay.
[b] 1/3 samples identified as HSV-1 negative by an alternative, FDA-cleared molecular assay.
[c] Initial invalid results are reported within the parentheses. The final number of invalid samples remaining after repeat testing are shown before the parenthesis.

Combined Sites: HSV-1 Mucocutaneous (N=710)

		Reference Method			*illumigene*	Performance			
		Pos	**Neg**	**Total**	**INV[d]**				**95% CI**
***illumigene* HSV 1&2**	**Pos**	152	28[b]	180	0 (1)	Sensitivity	152/160	95.0%	90.5-97.5%
	Neg	8[c]	522	530	0 (5)	Specificity	522/550	94.9%	92.7-96.5%
	Total	160	550	710	0 (6)[a]				

[a] There were six initial INV samples by ***illumi**gene*. Five repeated as ***illumi**gene* negative (ELVIS negative); one repeated as ***illumi**gene* HSV 1 positive (ELVIS HSV 1 positive).
[b] 19/28 samples identified as HSV-1 positive by an alternative, FDA-cleared molecular assay; 3 samples could not be tested.
[c] 7/8 samples were identified as HSV-1 negative by an alternative, FDA-cleared molecular assay; 1 sample could not be tested.
[d] Initial invalid results are reported within the parentheses. The final number of invalid samples remaining after repeat testing are shown before the parenthesis.

Combined Sites: HSV-2 Cutaneous (N=306)

		Reference Method			*illumigene*	Performance			
		Pos	**Neg**	**Total**	**INV[c]**				**95% CI**
***illumigene* HSV 1&2**	**Pos**	42	13[b]	55	0 (0)	Sensitivity	42/42	100%	91.6-100.0%
	Neg	0	251	251	0 (1)	Specificity	251/264	95.1%	91.8-97.1%
	Total	42	264	306	0 (1)[a]				

[a] There was one initial INV sample by ***illumi**gene*. The sample repeated as ***illumi**gene* negative (ELVIS negative).
[b] 8/13 samples were identified as HSV-2 positive by an alternative, FDA-cleared molecular assay; 1 sample could not be tested.
[c] Initial invalid results are reported within the parentheses. The final number of invalid samples remaining after repeat testing are shown before the parenthesis.

Combined Sites: HSV-2 Mucocutaneous (N=849)

		Reference Method			*illumigene*	Performance			
		Pos	**Neg**	**Total**	**INV[d]**				**95% CI**
***illumigene* HSV 1&2**	**Pos**	137	31[b]	168	0 (0)	Sensitivity	137/139	98.6%	94.9-99.6%
	Neg	2[c]	679	681	0 (1)	Specificity	679/710	95.6%	93.9-96.9%
	Total	139	710	849	0 (1)[a]				

[a] There was one initial INV sample by ***illumi**gene*. The sample repeated ***illumi**gene* negative (ELVIS negative).
[b] 24/31 samples were identified as HSV-2 positive by an alternative, FDA-cleared molecular assay; 4 samples could not be tested.
[c] 1/2 samples were identified as HSV-2 negative by an alternative, FDA-cleared molecular assay.
[d] Initial invalid results are reported within the parentheses. The final number of invalid samples remaining after repeat testing are shown before the parenthesis.

Prevalence of HSV-1 and HSV-2 in cutaneous and mucocutaneous lesion specimens was estimated for the 1158 specimens producing valid results with the ***illumi****gene* HSV 1&2 assay. One sample generated invalid results by ***illumi****gene* HSV 1 and HSV 2 and it was therefore excluded; this sample is also ELVIS indeterminate due to cell contamination. Two samples tested for HSV-1 and one sample tested for HSV-2 generated invalid results by ***illumi****gene* that were not repeated due to processing errors were also excluded. One sample unable to be typed by ELVIS and two HSV-1/HSV-2 coinfected specimens were included in the prevalence calculation as they produced valid ***illumi****gene* results. ELVIS HSV-2 positive samples, excluded from the HSV-1 performance calculation, were included in the prevalence calculation. Of the remaining eligible samples producing valid results, 306 were from cutaneous lesions; there were 849 specimens tested for HSV-1 and 850 specimens tested for HSV-2 from mucocutaneous lesions. The study population included specimens from pediatric, adult, and geriatric patients, with ages ranging from 1 day to 89 years. There were three samples tested for HSV-1 and two tested for HSV-2 mucocutaneous from patients with unknown age.

The prevalence of HSV-1 and HSV-2 by the ***illumi****gene* HSV 1&2 assay by anatomical location and patient age is provided in the tables below.

Prevalence by Anatomical Location (All Sites) – Cutaneous (N=306)

Location	HSV-1			HSV-2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Genital - Penis	92	7	7.6%	92	28	30.4%
Skin Lesion	214	47	22.0%	214	27	12.6%

Prevalence by Anatomical Location (All Sites) – Mucocutaneous

Location	HSV-1 (N=849)			HSV-2 (N=850)		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
Anorectal	47 (1*)	7	14.9%	46 (2*)	9	19.6%
Genital - Vaginal/Cervical	624 (2*)	112	17.9%	626	158	25.2%
Nasal	18	9	50.0%	18	0	0.0%
Ocular	20	0	0.0%	20	0	0.0%
Oral Lesion	135	54	40.0%	135	2	1.5%
Urethral	5	1	20.0%	5	0	0.0%

* Number of samples producing invalid ***illumi****gene* results, which could not be resolved and therefore, were excluded from the analysis.

Prevalence by Age (All Sites) – Cutaneous (N=306)

Age	HSV-1			HSV-2		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	38	12	31.6%	38	1	2.6%
6 to 11 years	14	7	50.0%	14	1	7.1%
12 to 21 years	51	14	27.5%	51	4	7.8%
22 to 59 years	166	18	10.8%	166	36	21.7%
≥60 years	37	3	8.1%	37	13	35.1%
Not Provided	0	0	0.0%	0	0	0.0%

Prevalence by Age (All Sites) – Mucocutaneous

Age	HSV-1 (N=849)			HSV-2 (N=850)		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 5 years	47	8	17.0%	47	0	0.0%
6 to 11 years	12	0	0.0%	12	0	0.0%
12 to 21 years	174 (1*)	46	26.4%	175	42	24.0%
22 to 59 years	550 (1*)	111	20.2%	551	116	21.1%
≥60 years	63 (1*)	18	28.6%	63 (1*)	11	17.5%
Not Provided	3	0	0.0%	2 (1*)	0	0.0%

* Number of samples producing invalid ***illumi****gene* results, which could not be resolved and therefore, were excluded from the analysis.

Expected Values:

The observed expected values in the clinical study are calculated using all eligible cutaneous and mucocutaneous lesion samples submitted for HSV testing. The overall incidence of HSV infection by the ***illumi****gene* HSV 1&2 assay during the clinical study is 20.5% (237/1155) for HSV-1 and 19.4% (224/1156) for HSV-2. Three HSV-1 and two HSV-2 samples producing invalid ***illumi****gene* results that could not be resolved were excluded from the total eligible sample population.

CONCLUSION

The ***illumi****gene*® HSV 1&2 DNA amplification assay, performed on the ***illumi****pro-10*™, can be used to detect HSV-1 and HSV-2 in cutaneous and mucocutaneous lesion specimens and is substantially equivalent to the predicate device.