510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K151046

B. Purpose for Submission:

To obtain substantial equivalence determination for the ***illumi****gene*® HSV 1&2 DNA Amplification Assay.

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:

Qualitative *in vitro* diagnostic device for the direct detection and differentiation of HSV-1 and HSV-2 DNA in cutaneous and mucocutaneous lesion specimens from symptomatic patients suspected of Herpetic infections.

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

illumi*gene*® HSV 1&2 DNA Amplification Assay

illumi*gene*® HSV 1&2 External Controls

G. Regulatory Information:

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

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Testing is performed on the ***illumi****pro-10*™ Automated Isothermal Amplification and Detection System from Meridian Bioscience, Inc.

I. 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 Control 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. The ***illumi****gene* HSV 1&2 kit includes the ***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 50 µL transfer pipettes. The specimen is added directly to the single-use SMP PREP III, which contains buffer and formalin-treated *E. coli* harboring *S. aureus DNA*. The *S. aureus* DNA serves as internal control DNA. A sample processed through the SMP PREP III is then heat-treated to make target and internal control DNA available for amplification. The heat-treated sample is added to each ***illumi****gene* HSV 1 and ***illumi****gene* HSV 2 Test Device. Mineral oil is added to each ***illumi****gene* Test Device to prevent evaporation.

The ***illumi****gene* HSV Test Device is a **t**wo-chambered device containing lyophilized amplification reagents (DNA polymerase, deoxynucleotide triphosphates) and either HSV-1 or 2-specific primers in the TEST chamber and *S. aureus*-specific primers in the CONTROL chamber**.** The ***illumi****gene* HSV 2 Test Device is visually identified by an orange band on the Test Device closure tab.

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 and internal control DNA. When HSV-1 or HSV-2 is present in the specimen, a 208 base pair sequence (bp) 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 a fixed cut-off value for disposition of results. Fixed cut-off values for the CONTROL chamber are used to determine validity. Fixed cut-off values for the TEST chamber are used to report sample results.

CONTROL chamber S_f:S_i ratios less than 90% are considered valid and allow for reporting of TEST chamber results. 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'. 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.

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 assist the user in detection of reagent deterioration, adverse environmental or test conditions, or variance in operator performance that may lead to test errors.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Lyra™ Direct HSV 1 + 2/VZV assay (Quidel Corporation)

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

   K133448

3. Comparison with predicate:

Similarities		
	DEVICE ***illumi*gene® HSV 1&2 DNA Amplification Assay** **K151046**	**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 infection.
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 andHSV-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** **K151046**	**PREDICATE** **Quidel** **Lyra™ Direct HSV 1 + 2/VZV Assay** **K133448**
Amplification Methodology	Loop-Mediated Isothermal Amplification (LAMP)	Multiplex Real-Time PCR
Detection Methodology	Turbidity	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

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

- Clinical and Laboratory Standards Institute, EP12-A2 (2008). User Protocol for Evaluation of Qualitative Test Performance.
- Clinical and Laboratory Standards Institute, EP17-A2 (2013). Protocols for determination of limits of detection and limits of quantitation.
- Clinical and Laboratory Standards Institute, EP07-A2 (2007). Interference Testing in Clinical Chemistry.
- CEN, EN-13640 (2004). Stability testing of *in vitro* diagnostic reagents.

L. Test Principle:

The ***illumi****gene* HSV 1&2 assay is based on loop mediated isothermal amplification technology (LAMP). Loop mediated amplification of DNA is accomplished by the use of specially designed primers that provide specific and continuous isothermal amplification. When HSV target DNA is present in the patient sample, the target sequence is amplified. Magnesium pyrophosphate, a by-product of DNA amplification, will reach saturation and precipitate thereby producing a turbid solution in the TEST chamber of the test device.

When sample preparation has been performed successfully and inhibition has not occurred, the internal control DNA in the CONTROL chamber of the test device will amplify. Magnesium pyrophosphate produced by the DNA amplification will precipitate and form a turbid solution in the CONTROL chamber.

The ***illumi****pro-10*™ instrument detects the change in light transmission through the reaction mixture created by precipitating magnesium pyrophosphate. Sample results are reported as Positive or Negative based on the detected change in light transmission. If there is a failure in the amplification of the internal control DNA in the CONTROL chamber, an Invalid report is provided.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility studies were performed at three ***illumi****gene* HSV 1&2 clinical sites. Studies were conducted using blind coded panels consisting of moderately positive (n=3), low positive (n=3), high negative (n=6) and negative (n=6) samples for each of HSV-1 and HSV-2. Contrived moderately positive, low positive and high negative samples were prepared by inoculating simulated negative matrix (cheek swab matrix in MicroTest™ M4® medium) with HSV-1 (strain HF) and HSV-2 (strain MS) to the final concentrations indicated in Table 1. The moderate and low positive samples for HSV-1 were used as the negative samples for HSV-2 and vice versa. Qualification of each sample included baseline testing in triplicate with ***illumi****gene* HSV 1&2 prior to storage at -70°C. The frozen samples were thawed and tested in twenty (20) replicates with each of three kit lots of ***illumi****gene* HSV 1&2 to qualify the samples according to the overall expected correlation in Table 1.

Table 1. Description of HSV-1 and HSV-2 Reproducibility Samples

Sample Type	Overall Expected Correlation HSV-1	Overall Expected Correlation HSV-2	HSV-1 Sample Concentration ($TCID_{50}$/mL)	HSV-2 Sample Concentration ($TCID_{50}$/mL)	Total Replicates
Moderate Positive HSV-1/ Negative HSV-2	100% Positive	100% Negative	2.16×10^4	0	90
Low Positive HSV-1/ Negative HSV-2	95% Positive	100% Negative	1.08×10^4	0	90
Moderate Positive HSV-2/ Negative HSV-1	100% Negative	100% Positive	0	4.8×10^3	90
Low Positive HSV-2/ Negative HSV-1	100% Negative	95% Positive	0	2.4×10^3	90
Near Cut-Off HSV 1 & 2	20-80% Negative	20-80% Negative	308	24	90
High Negative HSV 1 & 2	95% Negative	95% Negative	29.7	2.2	90
Reported Product LoD: HSV-1 (HF strain) = 7.20×10^3 $TCID_{50}$/mL; HSV-2 (MS strain) = 1.60×10^3 $TCID_{50}$/mL					

Three lots of ***illumi****gene* HSV 1&2 and five ***illumi****pro-10* instruments were used in the reproducibility studies. Positive and Negative Controls were tested with each panel. Each clinical site tested two panels each day for five days. At least two operators at each facility performed the testing to demonstrate reproducibility. The results from the reproducibility study are provided in Table 2 below:

Table 2. HSV-1 and HSV-2 Reproducibility

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[a]	20/30	66.7%	26/30	86.7%	20/30	66.7%	66/90	73.3%
HSV-1 & HSV-2 High Negative[b]	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-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[a]	25/30	83.3%	29/30	96.7%	25/30	83.3%	79/90	87.8%
HSV-1 & HSV-2 High Negative[b]	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%

[a] HSV-1: 308 $TCID_{50}$/mL, HSV-2: 24 $TCID_{50}$/mL
[b] HSV-1: 29.7 $TCID_{50}$/mL, HSV-2: 2.2 $TCID_{50}$/mL

The expected correlation for each sample type was achieved for all samples except the HSV-2 Near Cut-Off sample which demonstrated a higher overall negative correlation (87.8%) for all sites combined. By definition, the sample was designed to generate a positive or negative result with low probability (20-80%). Therefore, the sample type range is difficult to target with a qualitative assay such as the ***illumi****gene* HSV 1&2 assay. The High Negative sample was manufactured at a higher confidence range (95% negative) and met all expected correlation specifications, demonstrating reproducibility with a high negative sample. The studies demonstrate that the reproducibility of the ***illumi****gene* HSV 1&2 assay with the ***illumi****pro-10* is acceptable.

b. Linearity/assay reportable range:

Not applicable as the ***illumi****gene* HSV 1&2 DNA Amplification Assay is a qualitative assay.

c. Specimen Stability:

Specimen Stability

Sample stability was evaluated to determine transport and storage conditions prior to sample preparation. The study included testing of cheek swab matrix and vaginal swab matrix specimens stored in each viral transport medium to be evaluated. Three simulated negative samples and three combined HSV-1/HSV-2 low positive samples were each tested in triplicate for the baseline sample type (MicroTest™ M4® medium/polyester swab). One simulated negative sample and one combined HSV-1/HSV-2 low positive sample were tested in triplicate for each additional viral

transport medium. The viral transport media evaluated are summarized in Table 3.

Table 3. Viral Transport Media and Specimen Collection Kits

Viral transport medium	Volume	Swab Type	Manufacturer
MicroTest™ M4®	3 mL	Polyester; traditional-tipped, plastic shaft	Remel
MicroTest™ M4RT®	3 mL	Polyester; traditional-tipped, plastic shaft	Remel
MicroTest™ M6™	3 mL	FLOQSwab™ (flocked); traditional-tipped, plastic shaft	Remel
UniTranz-RT™ Transport System	3 mL	Flocked; regular tip, plastic shaft	Puritan
UTM™ Virus Collection	3 mL	Flocked; regular tip, plastic shaft	Copan
UTM / Quest V-C-M	3 mL	FLOQSwab™ (flocked); regular tip, plastic shaft	HealthLink
UVT	3 mL	Flocked; regular tip, plastic shaft	BD
ViraTrans™ VTM	2 mL	Polyester; traditional-tipped, plastic shaft *	Bartels
VTM	2 mL	Polyester; traditional-tipped, plastic shaft *	Hardy Diagnostics

* No swab was included in the transport kit, therefore the swab from the Remel MicroTest™ M4®kit was used.

Simulated negative matrix was prepared by inoculation of each viral transport medium with confirmed negative cheek or vaginal specimens collected with traditional-tipped flocked or polyester swabs. Contrived HSV-1/HSV-2 combined low positive samples were prepared by inoculating simulated negative matrix with HSV-1 strain MacIntyre (VR-539) and HSV-2 strain G (VR-734) to approximately 1.48 x 10^5 $TCID_{50}$/mL and 1.80 x 10^3 $TCID_{50}$ /mL, respectively, or 1.5X the limit of detection for the ***illumi****gene* HSV 1&2 kit used for the study.

Specimen stability was evaluated by storing prepared specimens at the upper temperature limit (32 ± 2°C; actual maximum: 30.9°C) or refrigerated (2-8°C) prior to initiation of ***illumi****gene* HSV 1&2 Specimen Preparation. Specimens stored at 32 ± 2°C were tested through a minimum of 25 hours and specimens stored refrigerated were tested through 8 days.

The results support the stability claim that specimens stored in viral transport medium may be held refrigerated (2-8°C) for up to 7 days prior to initiation of ***illumi****gene* HSV 1&2 Specimen Preparation.

Specimens stored at 32 ± 2°C for a minimum of 25 hours did not meet the acceptance criteria for all transport media. This temperature storage will not be included in the ***illumi****gene* HSV 1&2 DNA Amplification Assay Package Insert as an acceptable specimen storage condition.

Extracted Specimen Stability

Specimens diluted and processed through the ***illumi****gene* Sample Preparation Apparatus III (SMP PREP III) were evaluated for appropriate storage conditions prior to sample heat treatment and testing with the ***illumi****gene* HSV 1&2 DNA Amplification Assay. The study included testing of cheek swab matrix and vaginal swab matrix specimens stored in each viral transport medium to be evaluated. Three simulated negative specimens and three combined HSV-1/HSV-2 low positive specimens were tested in triplicate for the baseline sample type (MicroTest™ M4® medium/polyester swab). One simulated negative specimen and one combined HSV-1/HSV-2 low positive specimen were tested in triplicate for each additional viral transport medium. The viral transport media evaluated are the same as summarized in Table 3.

Simulated negative matrix was prepared by inoculation of each viral transport medium with confirmed negative cheek or vaginal specimens collected with traditional-tipped flocked or polyester swabs. Contrived HSV-1/HSV-2 combined low positive specimens were prepared by inoculating simulated negative matrix with HSV-1 strain MacIntyre (VR-539) and HSV-2 strain G (VR-734) to approximately 1.48 x 10^5 $TCID_{50}$/mL and 1.80 x 10^3 $TCID_{50}$/mL, respectively, or 1.5X the limit of detection for the ***illumi****gene* HSV 1&2 kit lot used for the study (LoD: HSV-1, 9.89 x 10^4 $TCID_{50}$/mL and HSV-2, 1.20 x 10^3 $TCID_{50}$/mL).

Specimens processed through SMP PREP III were stored at the upper temperature limit (32 ± 2°C; actual maximum: 30.9°C) or refrigerated (2-8°C) prior to initiation of heat treatment. Specimens stored at 32 ± 2°C and refrigerated were tested through at least 3 hours. External Positive and Negative Controls were tested daily. Specimens that produced unacceptable results were repeated by two technicians in duplicate.

The cheek and vaginal specimens processed through SMP PREP III and then stored at 32 ± 2°C produced acceptable results with storage up to 3 hours prior to heat treatment for all transport media. One contrived positive vaginal matrix sample prepared in ViraTrans VTM and stored refrigerated (2-8°C) for 3 hours produced unacceptable results with ***illumi****gene* HSV 1 only (1/3 replicates produced false negative results**).** Repeat testing confirmed the previous false negative results (4/4 false negative results).

As a result of the failure observed at 2-8°C storage with HSV-1 contrived positive vaginal matrix specimens prepared in ViraTrans VTM, additional testing was carried out to support the lower limit of the claimed 19-30°C range for processed specimen stability. The additional testing was performed with HSV-1 in vaginal matrix in ViraTrans VTM, MicroTest M4, and MicroTest M4RT at 17 ± 2°C).

The study results support storage of SMP PREP III processed specimens for up to 2 hours at 19-30°C prior to heat treatment.

d. Kit Stability

Data derived from accelerated stability studies support an initial shelf life claim of the test kit for 18 months at 2-30°C. Accelerated stability testing was performed with three lots of SMP PREP III, ***illumi****gene* HSV 1 Test Devices, and ***illumi****gene* HSV 2 Test Devices. Real-time stability studies are ongoing.

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

Calibrators

No calibrators are supplied or used with this assay.

Controls

External Controls: 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 and is sold separately from the ***illumi****gene* HSV 1&2 DNA Amplification Assay Kit. The external Positive Control contains non-infectious plasmid DNA with either HSV-1 or HSV-2 inserts. The external Negative Control consists of buffer. It is recommended that external controls are run with each new lot and new shipment of ***illumi****gene* HSV 1&2 kits. External control testing should be performed thereafter in accordance with appropriate federal, state and local guidelines. 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. Results from the ***illumi****gene* HSV 1&2 test kit should not be reported if external controls do not produce the expected results.

Storage Conditions: External Control reagents are intended to be stored at 2-8°C when not in use.

Stability: Accelerated stability testing of the external controls supports a stability claim of 18 months at 2-8°C. Accelerated stability testing was performed with three lots of Positive Control and Negative Control IV. Real time studies to confirm the shelf life claim are ongoing.

Traceability: Traceability to reference material does not apply to the External Controls. Controls are prepared from fully documented materials.

Validation: The performance of External Positive and Negative Controls were validated during the clinical trials.

Internal Control DNA: Primers for amplification of the internal control DNA are incorporated into each device in the CONTROL chamber. Internal control DNA is

present in the SMP PREP III buffer as formalin-treated *E. coli* cells harboring a plasmid carrying a segment of the *S. aureus* genome and is thus combined with each specimen and processed through all steps of the procedure. The internal control DNA is present to monitor for sample processing, amplification inhibition, and assay reagent integrity.

Storage Conditions: The ***illumi****gene* HSV 1&2 kit components are intended to be stored at 2-30°C when not in use.

Stability: The stability of the internal control DNA has been studied as part of the kit.

Traceability: Traceability to reference material does not apply to the SMP PREP III or Test Devices. Components are prepared from fully documented materials.

Validation: The performance of the ***illumi****gene* HSV 1&2 kit components, including the internal control DNA, was validated as part of the clinical trials.

f. 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 included the evaluation of characterized positive and negative clinical specimens. Amplification reagent concentrations were adjusted during design as needed to ensure that ***illumi****gene* HSV 1&2 assay results are aligned with clinical specimen reported results.

Cut-off values are applied as described in the Device Description section.

g. Detection limit (LoD):

Analytical Sensitivity studies were designed to determine the analytical limit of detection (LoD) of the ***illumi****gene* herpes simplex virus type 1 and type 2 DNA Amplification Assay. The LoD is defined as the lowest concentration of analyte ($TCID_{50}$/mL) that gives a 95% positivity rate.

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 (MicroTest™ M4® viral transport medium inoculated with HSV negative cheek swabs) were used to establish the LoD with three kit lots of ***illumi****gene* HSV 1&2 and six ***illumi****pro-10* instruments. A minimum of four dilutions near the expected LoD were evaluated for each HSV strain during preliminary testing of twenty (20) individually prepared replicates. Replicates for each dilution were tested with the ***illumi****gene* HSV 1&2 DNA Amplification Assay; however, testing for a specific concentration was discontinued

when more than one negative result was obtained. The lowest dilution producing positive results in a minimum of 19 of 20 replicates was identified as the tentative LoD. The tentative LoD for HSV-1was determined to be 7.20 x 10^3 $TCID_{50}$/mL for the HF Strain (VR-260) and 9.89 x 10^4 $TCID_{50}$/mL for the MacIntyre Strain (VR-539). For HSV-2, the tentative LoD was 1.20 x 10^3 $TCID_{50}$/mL for the G Strain (VR-734) and 533 $TCID_{50}$/mL for the MS Strain (VR-540).

Confirmatory LoD studies were done through testing of an additional 60 individually prepared replicates at the identified preliminary LoD for each strain. The target dilutions were prepared by two technicians who did not prepare the original dilution series. If the selected target dilution did not meet acceptance criteria for LoD (minimum of 57/60 positive), the next dilution containing a greater concentration of measurand was prepared and tested in 60 replicates. All testing was performed using three kit lots of ***illumi****gene* HSV 1&2 and six ***illumi****pro-10* instruments.

External Positive and Negative Controls were tested daily during preliminary and final LoD assessment by each technician for each kit lot.

The overall confirmed analytical LoD by HSV strain for ***illumi****gene* HSV 1&2 is summarized below:

Table 4. Overall *illumi*gene HSV 1&2 Confirmed Analytical Limit of Detect

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

The final confirmed LoD for the ***illumi****gene* HSV 1&2 Assay is 9.89 x 10^4 $TCID_{50}$/mL for HSV-1 and 1.60 x 10^3 $TCID_{50}$/mL for HSV-2.

h. Analytical Reactivity:

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 specimens from a variety of anatomical locations (i.e. oral, genital, anorectal, etc.) at the assay LoD. Each clinical sample was quantified and diluted in the corresponding viral transport medium to 9.89 x 10^4 $TCID_{50}$/mL or 1.60 x 10^3 $TCID_{50}$/mL for HSV-1 and HSV-2, respectively; specimens quantified at concentrations less than the limit of detection were tested undiluted. All specimens were detected by ***illumi****gene* HSV 1&2 at LoD or lower, except one HSV-1 clinical sample, which was detected at 7.20 x 10^7 $TCID_{50}$/mL.

i. Analytical specificity/ Cross-Reactivity:

Cross-reactivity studies were performed to determine if potential microbial co-contaminants of cutaneous and mucocutaneous lesion swab specimens react with the ***illumi****gene* HSV 1&2 DNA Amplification Assay. Potential interference of the ***illumi****gene* HSV 1&2 DNA Amplification Assay with these microbial organisms was also evaluated.

Simulated negative samples were prepared by vortexing traditional-tipped polyester swabs in MicroTest™ M4® viral transport medium. Contrived HSV-1/HSV-2 combined low positive samples were prepared by inoculating simulated negative matrix with HSV-1 (strain HF) and HSV-2 (strain MS) to approximately 1.08 x 10^4 $TCID_{50}$/mL and 2.40 x 10^3 $TCID_{50}$/mL, respectively, or 1.5X the limit of detection (LoD) for these strains.

Potentially cross-reactive microorganisms were added to simulated negative and contrived positive samples at minimum concentrations of 1.0 x 10^6 CFU/mL (bacteria and fungi) or 1.0 x 10^5 $TCID_{50}$/mL or comparable units (viruses). Quantified genomic DNA or RNA used for microorganisms and human genomic DNA was tested at 1.0 x 10^6 copies/mL (cp/mL) or higher. Negative and positive samples spiked with potential cross-reacting microorganisms were tested in triplicate.

External Positive and Negative Controls were tested daily. Dilution Controls were prepared by adding 0.85% saline or Tris-EDTA buffer in place of the potentially cross-reactive organisms or genetic material. Matrix Controls (prepared negative and positive matrix alone) and Dilution Controls were tested in triplicate.

None of the following organisms or their genetic material reacted with the ***illumi****gene* HSV 1&2 DNA Amplification Assay:

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.

j. Interference Studies:

Interference Substances:

A panel of 23 chemical and seven biological non-microbial contaminants, representing substances expected to be found in cutaneous or mucocutaneous lesions, was evaluated for the potential to interfere with the ***illumi****gene* HSV 1&2 assay. The potentially interfering substances were tested with one simulated negative sample (N1) and two unique contrived HSV-1/HSV-2 low positive samples prepared with HSV-1 (strains HF and MacIntyre) and HSV-2 (strains G and MS).

Simulated negative matrix (N1) was prepared by vortexing traditional-tipped polyester swabs in Remel MicroTest™ M4 viral transport media.

Contrived HSV-1/HSV-2 combined low positive samples were prepared by inoculating simulated negative matrix with quantified HSV-1 and HSV-2 stocks to approximately 1.5X LoD.

Positive sample P1 was prepared using HSV-1 (strain MacIntyre) and HSV-2 (strain G) at final concentrations of 1.48 x 10^5 $TCID_{50}$/mL and 1.80 x 10^3 $TCID_{50}$/mL, respectively.

Positive sample P2 was prepared using HSV-1 (strain HF) and HSV-2 (strain MS) at final concentrations of 1.08 x 10^4 $TCID_{50}$/mL and 2.40 x 10^3 $TCID_{50}$/mL, respectively.

Potentially interfering substances were then added to negative and positive samples at final concentrations of 60 µg/mL, 7% v/v, 7% w/v, or greater (final concentrations are provided in the data summary Table 5).

The prepared samples, Matrix Controls, and Dilution Controls were tested in triplicate. Replicates producing unexpected results were further evaluated in

replicates of ten for N1, P1 and P2 matrices.

Cold-EEZE® (7% v/v) produced unexpected results (3/3 invalid results) during original testing of both negative and positive specimens. Repeat testing with 10 replicates for each sample type (N1, P1, P2) produced invalid results for all replicates.

Casein at concentrations greater than 5 mg/mL was found to interfere with the assay, producing false negative results.

Table 5. Potentially Interfering Substances

Interferent / Active Ingredient(s)	Final Concentration Tested	Interference Observed (X = yes)
Abreva® Docosanol, 10%	7% w/v	
Acetaminophen	5 mg/mL	
Acetylsalicylic acid	10 mg/mL	
Acyclovir	7 mg/mL	
Balneol® Hygienic Cleansing Lotion	7% w/v	
Carmex® Original Lip Balm Camphor, 1.7%; Menthol, 0.7%	7% w/v	
Cold-EEZE® Cold Remedy plus Sore Throat Zincum Gluconicum 2X	7% v/v	X
Cornstarch	1.25 mg/mL	
Crest® Complete Fluoride Toothpaste Sodium fluoride, 0.243%	7% w/v	
Chlorpheniramine maleate	5 mg/mL	
Dextromethorphan hydrobromide	10 mg/mL	
CVS Pharmacy® Disposable Douche	7% v/v	
CVS™ Yeast Infection Relief Miconazole 3 Miconazole nitrate, 2%	7% w/v	
Desitin® Zinc Oxide, 40%	7% w/v	
K-Y® Brand Jelly	7% w/v	
Lanacane® Benzethonium chloride, 0.2%; Benzocaine, 20%	7% v/v	
Lip Clear® Lysine+® Zinc Oxide, 1.2%	7% w/v	
Listerine® Original Eucalyptol, 0.092%; Menthol, 0.042%; Methyl salicylate, 0.060%; Thymol, 0.064%	7% v/v	
Preparation H® Hemorrhoidal Ointment Mineral oil, 14%; Petrolatum, 74.9%; Phenylephrine HCl, 0.25%	7% w/v	
Releev® Benzalkonium chloride, 0.13%	7% v/v	
Tioconazole	7% w/v	
Vagisil® Regular Strength	7% w/v	

Interferent / Active Ingredient(s)	Final Concentration Tested	Interference Observed (X = yes)
Benzocaine, 5%; Resorcinol 2%		
Yeast Gard® Gel Treatment Candida albicans, 27X HPUS; Candida parapsilosis, 27X HPUS; Pulsatilla, 27X HPUS	7%w/v	
Albumin	3.3 mg/mL	
Casein	7 mg/mL	X
	8 mg/mL	X
	6 mg/mL	X
	5 mg/mL	
Feces	7% w/v	
Mucus (Mucin, bovine submaxillary gland, type I-S)	60 µg/mL	
Seminal fluid	7% v/v	
Urine	7% v/v	
Whole blood	7% v/v	
Buffy coat	7% v/v	

Viral Transport media:

An equivalency study was performed to confirm the suitability of viral transport medium and specimen collection kits commonly used for cutaneous and mucocutaneous lesion specimen collection and transport. The viral transport media and specimen collection kits evaluated in the study were the same as those summarized in Table 3. One negative sample and one HSV-1/HSV-2 combined low positive sample were evaluated with each viral transport medium collection kit.

Negative matrix was prepared by vortexing each swab in the corresponding viral transport medium. Contrived low positive samples were prepared through inoculation of negative matrix with HSV-1 (strain HF) and HSV-2 (strain MS) to approximately 1.5X LoD. Each combined HSV-1/HSV-2 low positive sample was prepared at final concentrations of 1.08×10^4 $TCID_{50}$/mL and 2.40×10^3 $TCID_{50}$/mL, respectively. The collection device and viral transport medium in the HealthLink UTM collection kit were considered to be equivalent to Quest V-C-M; therefore, only HealthLink UTM was evaluated as a representative sample type for both viral transport media collection kits.

Negative samples were tested in triplicate and positive samples were tested in replicates of twenty.

All negative and positive replicates were correctly detected. The results confirmed the equivalence of all viral transport media collection kits evaluated.

Viral transport media containing protein stabilizers, such as Remel MicroTest™ M5® and calcium alginate swabs, were previously identified to impact performance

of the ***illumi****gene* HSV 1&2 assay and therefore were not evaluated as part of this study and are indicated in the Package Insert as not acceptable for use with the ***illumi****gene* HSV 1&2 assay.

k. Carry-over/ Cross Contamination:

Analysis of the ***illumi****gene* HSV 1&2 assay design, procedure, sample preparation equipment, Test Devices, and user interface demonstrates that the risk of carry-over is comparable to the previously cleared ***illumi****gene* assays.

Carry-over studies previously completed for ***illumi****gene* Mycoplasma (K123432), ***illumi****gene* Pertussis (K133673), and ***illumi****gene* Group A *Streptococcus* (K122019) have demonstrated that ***illumi****gene* sample preparation equipment and test devices do not contribute to cross-contamination between samples.

2. Comparison studies:

a. Method comparison with predicate device: Not Applicable. Refer to the Clinical section below.

b. Matrix comparison: Not Applicable.

3. Clinical studies:

a. Clinical Sensitivity: Not Applicable.

b. Clinical specificity: Not Applicable.

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

Clinical Performance

The performance of the ***illumi****gene* HSV 1&2 DNA Amplification Assay with cutaneous (*e.g.,* skin lesion, genital (penis)) or mucocutaneous (*e.g.,* anorectal, genital (vaginal/cervical), nasal, ocular, oral lesion and urethral) specimens was determined from testing of 1,158 eligible specimens collected in seven clinical sites. The performance of the ***illumi****gene* HSV 1&2 DNA Amplification Assay was compared with the ELVIS® HSV ID/Typing Test System (ELVIS), 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.

Specimens were excluded in the performance analysis due to ELVIS cell contamination (n=1), inability to be HSV-typed by the reference ELVIS method (n=1), or invalid results by the ***illumi****gene* HSV 1&2 DNA Amplification Assay without repeat testing (n=1 for HSV-1 and n=1 for HSV-2). Finally, samples which gave HSV-2 positive results from the ELVIS method were excluded from the HSV-1 performance calculation because of the inability of the ELVIS method to distinguish

an HSV-1 positive sample when HSV-2 was detected first (n=181).

In summary, 974 HSV-1 and 1155 HSV-2 specimen results were included in the performance analysis of the ***illumi****gene* HSV 1&2 DNA Amplification Assay versus the ELVIS reference method.

A total of 723 (74.3%) female and 247 (25.3%) male specimens were tested for HSV-1 and a total of 873 (75.6%) female and 277 (24.0%) male specimens were tested for HSV-2. There were four (4) specimens tested for HSV-1 (0.4%) and five (5) tested for HSV-2 specimens with unknown gender (0.4%). There is no expectation that assay performance is influenced by gender.

For HSV-1 cutaneous specimens, there were 264 valid results with the ELVIS method. No invalid results were generated by the ***illumi****gene* HSV 1&2 DNA Amplification Assay.

For HSV-1 mucocutaneous specimens, there were 710 valid results with the ELVIS method with six initially invalid results by the ***illumi****gene* HSV 1&2 DNA Amplification Assay. All initially invalid results were resolved by repeat testing as per the Package Insert and included in the performance calculation.

For HSV-2 cutaneous specimens, there were 306 valid results with the ELVIS method with one initially invalid result by the ***illumi****gene* HSV 1&2 DNA Amplification Assay. The initially invalid result was resolved by repeat testing and included in the performance calculation.

For HSV-2 mucocutaneous specimens, there were 849 valid results with the ELVIS method with one originally invalid result by the ***illumi****gene* HSV 1&2 DNA Amplification Assay. The initially invalid results was resolved and included in the performance calculation.

The performance of the ***illumi****gene* HSV 1&2 DNA Amplification Assay against the ELVIS reference method is presented in Table 6 below:

Table 6. Clinical Performance

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

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

[a] 6/6 specimens identified as HSV-1 positive by an alternative, FDA-cleared molecular assay.
[b] 1/3 specimens 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 specimens remaining after repeat testing is shown before the parenthesis.

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

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

[a] There were six initial INV specimens 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 specimens identified as HSV-1 positive by an alternative, FDA-cleared molecular assay; 3 specimens could not be tested.
[c] 7/8 specimens 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 specimens remaining after repeat testing is shown before the parenthesis.

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

		Reference Method			*illumi-gene*	Performance			
		Pos	**Neg**	**Total**	**INV**[c]				**95% CI**
***illumi*gene HSV 1&2**	**Pos**	42	13[b]	55	0 (0)	Sens	42/42	100%	91.6-100.0%
	Neg	0	251	251	0 (1)	Spec	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 specimens 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 specimens remaining after repeat testing is shown before the parenthesis.

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

		Reference Method			*illumi-gene*	Performance			
		Pos	**Neg**	**Total**	**INV**[d]				**95% CI**
***illumi*gene HSV 1&2**	**Pos**	137	31[b]	168	0 (0)	Sens	137/139	98.6%	94.9-99.6%
	Neg	2[c]	679	681	0 (1)	Spec	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 specimens were identified as HSV-2 positive by an alternative, FDA-cleared molecular assay; 4 specimens could not be tested.
[c] 1/2 specimens 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 specimens remaining after repeat testing is shown before the parenthesis.

4. Clinical cut-off: Not applicable.

5. Expected values/Reference range:

   The observed expected values in the clinical study were calculated using all eligible cutaneous and mucocutaneous lesion specimens submitted for HSV testing that gave valid ***illumi****gene* HSV 1&2 assay results. Three HSV-1 and two HSV-2 specimens producing invalid ***illumi****gene* results that could not be resolved were excluded from the 1158 total eligible sample population. Therefore, the total number of tested specimens that were included in the prevalence calculation were N=1155 for HSV-1 and N=1156 for HSV-2. The overall incidence of HSV infection by the ***illumi****gene* HSV 1&2 assay during the clinical study was 20.5% (237/1155) for HSV-1 and 19.4% (224/1156) for HSV-2.

   The prevalence of HSV-1 and HSV-2 with the ***illumi****gene* HSV 1&2 DNA Amplification Assay was calculated for the combined sites based on the specific source of specimen and the age of the patient.

   Of the eligible specimens, 306 were from cutaneous lesions. 849 were mucocutaneous specimens tested for HSV-1 and 850 were mucocutaneous specimens tested for HSV-2. There were three specimens tested for HSV-1 and two tested for HSV-2 mucocutaneous from patients with unknown age. The study population included specimens from pediatric, adult, and geriatric patients, with ages ranging from 1 day to 89 years.

   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

Location	HSV-1 N=306			HSV-2 N=306		
	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 specimens producing invalid ***illumi****gene* result, which could not be resolved and therefore, were excluded from the analysis.

Prevalence by Age (All Sites) – Cutaneous

Age	HSV-1 N=306			HSV-2 N=306		
	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 specimens producing invalid ***illumi****gene* result, which could not be resolved and therefore, were excluded from the analysis.

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.