510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K160829

B. Purpose for Submission:

The purpose of the 510(k) submission is to obtain a substantial equivalence determination for the ***illumi****gene* Mycoplasma Direct DNA Amplification Assay.

C. Measurand:

DNA target sequence is a 353-560 base pair (208 bp) region of an intracellular protease-like gene from *Mycoplasma pneumoniae*

D. Type of Test:

The ***illumi****gene*® Mycoplasma Direct DNA amplification assay is a qualitative in vitro diagnostic device for the direct detection of *Mycoplasma pneumoniae* in human throat swabs collected from patients suspected of having *Mycoplasma pneumoniae* infection. The molecular assay uses isothermal loop-mediated amplification (LAMP) technology that targets an intracellular protease-like gene of the *Mycoplasma pneumoniae* genome.

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

illumi*gene*® Mycoplasma Direct DNA Amplification Assay

illumi*gene*® Mycoplasma Direct External Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980: Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:

   Class II

3. Product code:

   OZX - *Mycoplasma pneumoniae* DNA Assay System

   OOI – Real Time Nucleic Acid Amplification System

4. Panel:

   Microbiology (83)

H. Intended Use/Indications for Use:

1. Intended Use/Indications for Use:

   The ***illumi****gene* Mycoplasma Direct DNA amplification assay, performed on the ***illumi****pro-10*™, is a qualitative in vitro diagnostic test for the direct detection of DNA from *Mycoplasma pneumoniae* in human throat swabs obtained from patients suspected of having *Mycoplasma pneumoniae* infection.

   The ***illumi****gene* Mycoplasma Direct assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect *Mycoplasma pneumoniae* by targeting a segment of the *Mycoplasma pneumoniae* genome.

   Results from the ***illumi****gene* Mycoplasma Direct DNA amplification assay should be used in conjunction with clinical presentation, other laboratory findings, and epidemiological risk factors as an aid in the diagnosis of *Mycoplasma* infection and should not be used as the sole basis for treatment or other patient management. Positive results do not rule out co-infection with other organisms and negative results in persons with respiratory tract infections may be due to pathogens not detected by this assay. Lower respiratory tract infections due to *M. pneumoniae* may not be detected by this assay. If lower respiratory tract infection due to *M. pneumoniae* is suspected, additional laboratory testing using methods other than the ***illumi****gene* Mycoplasma Direct DNA Amplification Assay may be necessary.

2. Special conditions for use statement(s):

   For Prescription Use Only

3. Special instrument requirements:

   illumi*pro-10*™ Automated Isothermal Amplification and Detection System from Meridian Bioscience, Inc.

I. Device Description:

The ***illumi****gene* Molecular Diagnostic Test System incorporates the ***illumi****gene*® Mycoplasma

Direct DNA Amplification Assay Test kit, the ***illumi****gene* Mycoplasma Direct External Controls kit, and the ***illumi****pro-10*™ Automated Isothermal Amplification and Detection System.

The ***illumi****gene* Mycoplasma Direct assay utilizes loop-mediated isothermal amplification (LAMP) technology to detect the presence of *Mycoplasma pneumoniae* in human throat swab specimens. The ***illumi****gene* Mycoplasma Direct assay is performed using a negative control (***illumi****gene* Sample Preparation Apparatus II/Negative Control III [SMP PREP II]), ***illumi****gene* Heat Treatment Tubes, and an ***illumi****gene* Mycoplasma Test Device. Collected throat swabs are placed directly into the SMP PREP II that contains assay control buffer. The specimen/control samples are processed through SMP PREP II and extracted DNA is heat-treated. Therefore, heat-treatment produces target and control DNA for isothermal amplification. The heat-treated specimen/control samples are added to the ***illumi****gene* Mycoplasma Test Device where DNA amplification occurs.

The ***illumi****pro-10*™ (Incubator/Reader) incubates each ***illumi****gene* Mycoplasma Test Device containing prepared sample and control material to 63°C that facilitates isothermal amplification of target DNA. When *M. pneumoniae* is present in the prepared specimen, a 208 base pair region of the *M. pneumoniae* genome is amplified and magnesium pyrophosphate is produced as a by-product of the reaction. Magnesium pyrophosphate forms a precipitate in the reaction mixture.

The ***illumi****pro-10*™ monitors the absorbance characteristics (visible light transmission) of each reaction solution 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 ratio of the Run End absorbance ($Signal_{final}$ or S_f) with the Run Start absorbance ($Signal_{initial}$, S_i) and compares the ratio (S_f:S_i) to an established cut-off value. These ratios are calculated by the ***illumi****pro-10*™ for both the TEST and CONTROL chambers of the device.

The ***illumi****gene* Mycoplasma External Control Kit contains a Positive control reagent for use in routine quality control testing and is provided separately from the Mycoplasma Direct Kit. The Sample Preparation Apparatus II/Negative Control III (SMP PREP) reagent is included with the Mycoplasma Direct Kit. The Sample Preparation Apparatus II contains a buffered solution with formalin treated *E. coli* containing *Staphylococcus aureus* DNA and is processed as the assay's internal control. The internal control monitors amplification inhibition, assay reagent performance and sample processing effectiveness. The Negative Control III component of SMP PREP functions as an External Negative Control. The Negative Control aids 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):
   illumi*gene*® Mycoplasma DNA Amplification Assay

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

K123423, K152800

3. Comparison with predicate:

Similarities		
Item	**Device *illumigene*® Mycoplasma Direct DNA Amplification Assay (K160829)**	**Predicate *illumigene*® Mycoplasma DNA Amplification Assay (K123423, K152800)**
Organisms Detected	*Mycoplasma pneumoniae*	*Mycoplasma pneumoniae*
Amplification and Detection	Self-contained and automated	Self-contained and automated
Analyte	DNA	DNA
Test Format	Automated; qualitative	Automated; qualitative
Assay Target	208 bp DNA sequence	208 bp DNA sequence
Instrumentation	***illumi****pro-10*™ Automated Isothermal Amplification and Detection System	***illumi****pro-10*™ Automated Isothermal Amplification and Detection System
Reading Method	Visible light transmission	Visible light transmission

Differences		
Item	**Device *illumigene*® Mycoplasma Direct DNA Amplification Assay (K160829)**	**Predicate *illumigene*® Mycoplasma DNA Amplification Assay (K123423, K152800)**
Intended Use/Indications for Use	The ***illumi****gene* Mycoplasma Direct DNA amplification assay, performed on the ***illumi****pro-10*™, is a qualitative in vitro diagnostic test for the direct detection of DNA from *Mycoplasma pneumoniae* in **human throat swabs** obtained from patients suspected of having *Mycoplasma pneumoniae* infection. The ***illumi****gene* Mycoplasma Direct assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect *Mycoplasma pneumoniae* by targeting a segment of the *Mycoplasma pneumoniae* genome.	The ***illumi****gene* Mycoplasma DNA amplification assay, performed on the ***illumi****pro-10*™, is a qualitative in vitro diagnostic test for the direct detection of DNA from *Mycoplasma pneumoniae* in **human throat and nasopharyngeal swabs** obtained from patients suspected of having *Mycoplasma pneumoniae* infection. The ***illumi****gene* Mycoplasma assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect *Mycoplasma pneumoniae* by targeting a segment of the *Mycoplasma pneumoniae*

Differences		
Item	**Device *illumigene*® Mycoplasma Direct DNA Amplification Assay (K160829)**	**Predicate *illumigene*® Mycoplasma DNA Amplification Assay (K123423, K152800)**
	Results from the ***illumi****gene* Mycoplasma Direct DNA amplification assay should be used in conjunction with clinical presentation, other laboratory findings, and epidemiological risk factors as an aid in the diagnosis of Mycoplasma infection and should not be used as the sole basis for treatment or other patient management. Positive results do not rule out co-infection with other organisms and negative results in persons with respiratory tract infections may be due to pathogens not detected by this assay. Lower respiratory tract infections due to *M. pneumoniae* may not be detected by this assay. If lower respiratory tract infection due to *M. pneumoniae* is suspected, additional laboratory testing using methods other than the ***illumi****gene* Mycoplasma Direct DNA Amplification Assay may be necessary.	genome. Results from the ***illumi****gene* Mycoplasma DNA amplification assay should be used in conjunction with clinical presentation, other laboratory findings, and epidemiological risk factors as an aid in the diagnosis of Mycoplasma infection and should not be used as the sole basis for treatment or other patient management. Positive results do not rule out co-infection with other organisms and negative results in persons with respiratory tract infections may be due to pathogens not detected by this assay. Lower respiratory tract infections due to *M. pneumoniae* may not be detected by this assay. If lower respiratory tract infection due to *M. pneumoniae* is suspected, additional laboratory testing using methods other than the ***illumi****gene* Mycoplasma DNA Amplification Assay may be necessary. ***illumi****gene* Mycoplasma is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.
Specimen Types	Human throat swabs	Human throat and nasopharyngeal swabs

Differences		
Item	**Device *illumigene*® Mycoplasma Direct DNA Amplification Assay (K160829)**	**Predicate *illumigene*® Mycoplasma DNA Amplification Assay (K123423, K152800)**
Swab Types	Rayon, Flocked Nylon, or Polyester	Cotton, Foam, Flocked Nylon, Polyester, or Rayon
Transport Media	Liquid Amies without charcoal or Liquid Stuart	0.85% saline, M4, M4-RT, M5, UTM-RT, or Liquid Amies without charcoal
Sample Processing/Preparation	Extraction reagents included with kit	Manual extraction with Qiagen® DSP DNA Minikit
External Controls	Positive control is provided separately in the ***illumi****gene* Mycoplasma Direct External Control Kit; External Negative Control is part of the Direct kit (Sample Preparation Apparatus II/Negative Control III) External Positive Control is a tris-buffered solution with plasmid target DNA; External Negative Control is formalin-treated *E. coli* with *S. aureus* DNA	External Positive and Negative Controls are provided separately in the ***illumi****gene* Mycoplasma External Control kit External Positive Control is a Tris-buffered solution with plasmid target DNA; External Negative Control is a tris-buffered solution with plasmid DNA carrying *S. aureus* insert
Kit Storage	2-27°C	2-8°C

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

None

L. Test Principle:

The ***illumi****gene* Mycoplasma Direct assay uses loop-mediated amplification (LAMP) technology that employs specially designed loop primers for specific and continuous isothermal amplification. When the target DNA is present in the specimen, the sequence is amplified and magnesium pyrophosphate is produced as a by-product. The magnesium pyrophosphate forms a white precipitate and causes the reaction solution to appear turbid. If there is a change in sample absorbance between Run End and Run Start due to magnesium pyrophosphate precipitation, then the target DNA is detected and the sample is considered positive. When the target DNA is absent, there is no detectable change in sample absorbance and the sample is considered negative. Sample readouts are determined by comparing the initial to final absorbance reading ratios to a fixed cut-off value.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Blind coded panels of 10 samples were provided to three participating clinical laboratory sites. Samples were randomly sorted within each panel to mask the sample identities. Each panel consisted of three moderate positive samples, three low positive samples, three high negative samples, and one negative sample. Contrived moderate positive, low positive, and high negative panel members were prepared using inoculated rayon swabs with simulated negative matrix (simulated throat swab matrix in Liquid Amies medium) using *M. pneumoniae* strain M129 at final concentrations provided in table below.

Sample ID	Final Concentration (CFU/Test)	Final Concentration (CFU/mL)	Expected Result
Moderate Positive	3.08	800	Positive
Low Positive	1.54	400	Positive
High Negative	0.002	0.519	Negative
Negative	0	0	Negative

Reported Product LoD (CFU/mL): 200 CFU/mL, which is equivalent to approximately 0.77CFU/Test

Three lots of ***illumi****gene* Mycoplasma Direct reagents, three lots of ***illumi****gene* Mycoplasma Direct External Controls, and eight ***illumi****pro-10*™ instruments were used in reproducibility studies. Each clinical site tested two panels each day for five days (inter-assay variability) with testing performed by two operators at each site on the same day (intra-assay variability). Positive and Negative Controls were tested each day of testing. Testing results are provided in the table below:

	Site 1		Site 2		Site 3		Total – All Sites				
Sample Type	**Percent Agreement**		**Percent agreement**		**Percent agreement**		**Percent agreement**		**Average S_f:S_i**	**SD**	**%SC**
High Negative	30/30	100.0%	30/30	100.0%	30/30	100%	90/90	100.0%	101.45	2.17	2.14
Low Positive	30/30	100.0%	30/30	100.0%	29/30	96.7%	89/90	98.9%	62.35	5.21	8.36
Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%	61.47	3.88	3.88
Negative	9/10	90.0%	10/10	100.0%	10/10	100.0%	29/30	96.7%	99.70	7.67	7.69
Negative Control	10/10	100.0%	10/10	100.0%	10/10	100.0%	30/30	100.0%	101.7	1.80	1.77
Positive Control	10/10	100.0%	10/10	100.0%	10/10	100.0%	30/30	100.0%	60.85	2.36	3.88

b. Linearity/assay reportable range:

Not applicable as the ***illumi****gene* Mycoplasma DNA amplification assay is a qualitative assay.

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

Specimen Stability:

Specimen storage and hold time parameters were evaluated for the ***illumi****gene* Mycoplasma Direct DNA amplification assay. Validation studies performed at Meridian were completed using rayon, polyester, or flocked nylon swabs with Liquid Amies without charcoal and Liquid Stuart. Samples were prepared using a simulated throat swab matrix spiked with *M. pneumoniae* strain M129 at high negative and low positive concentrations (200 CFU/mL).

Testing was performed in triplicate for each of the following storage conditions: (1) upper limit of room temperature (31 ± 2°C), (2) refrigerated (2-8°C), or (3) frozen (-28 to -16°C). Samples stored at room temperature were tested through 49+ hours and samples stored refrigerated or frozen were tested through 15 days. Results of the study supported that throat swabs (rayon, polyester, flocked nylon) in the above mentioned transport media can be held at the following claimed storage conditions: room temperature for up to 24 hours and refrigeration for up to 14 hours prior to sample preparation. Frozen storage is not part of the Mycoplasma Direct Package Insert and was only evaluated to support this mechanism of storage for analytical and clinical studies.

Extracted Specimen Stability:

Studies were performed to evaluate storage conditions of processed samples prior to testing with the ***illumi****gene* Mycoplasma Direct DNA amplification assay. Samples consisting of rayon, polyester, or flocked nylon swabs with Liquid Amies (without charcoal) or Liquid Stuart in a simulated negative matrix were spiked with *M. pneumoniae* M129. The prepared samples were held at room temperature (31 ± 2°C) for up to 3 hours and then heat-treated again for 10 minutes preceding testing on the ***illumi****pro-10*™. Results of the study support the claimed stability of heat-treated samples for up to 3 hours at room temperature (19-29°C). Heat treatment must be re-performed (95 ± 5°C for 10 ± 2 minutes) if heat-treated samples are stored prior to ***illumi****pro-10*™ testing.

Controls:

External Controls: The ***illumi****gene* Mycoplasma Direct Positive External Control Reagent includes an External Positive Control and is sold separately from the ***illumi****gene* Mycoplasma Direct DNA amplification assay. The External Positive Control contains non-infectious plasmid DNA with a *M. pneumoniae* insert that is

stored in a tris-buffered solution with azide. The Negative Control is a component of the Sample Preparation Apparatus II/Negative Control III reagent that is included in the ***illumi****gene* Mycoplasma Direct kit. The Negative External Control contains formalin-treated *E. coli* with *S. aureus* DNA. External Controls are necessary for routine Quality Control that help the end-user detect reagent deterioration, adverse environmental or test conditions, or differences in operator performance that result in test errors. Testing of external controls was performed for all analytical and clinical studies with no discordant results. It is recommended that external controls are run with each new lot and new shipment of ***illumi****gene* Mycoplasma Direct kits. External control testing should be performed thereafter in accordance with appropriate federal, state, and local guidelines. Results from the ***illumi****gene* Mycoplasma Direct kit should not be reported if external controls do not produce the expected results.

Internal Control: Each test device contains lyophilized amplification reagents and primers specific to the Internal Control and is housed in the CONTROL chamber. Internal Control DNA (tris-buffered solution with formalin-treated *E. coli* containing a plasmid with a segment of the *S. aureus* genome) is in the Sample Preparation Apparatus II (SMP PREP II) reagent and is processed through all steps of the test procedure. SMP PREP II is a built-in component of the ***illumi****gene* Mycoplasma Direct amplification assay. A functioning Internal Control is demonstrated by a valid assay result from the TEST chamber.

d. Detection limit:

Analytical sensitivity studies were designed to evaluate the analytical limit of detection (LoD) of the ***illumi****gene* Mycoplasma Direct DNA amplification assay. The LoD is the lowest number of colony forming units (CFUs) per test aliquot that can be distinguished from negative samples with a high degree of probability (95%). *M. pneumoniae* strains M129 and FH were used to establish the LoD. These strains were diluted in a simulated negative throat swab matrix (Liquid Amies with a rayon swab inoculated with throat gargle screened negative for *M. pneumoniae*). A minimum of three dilutions near the expected LoD in 20 individually prepared replicates were evaluated for each *M. pneumoniae* strain. Testing was performed using three lots of reagents and eight ***illumi****pro-10*™ instruments over multiple days with two different technicians. External Positive and Negative Controls were tested each day throughout the study. These studies confirmed that the LoD for the assay was 2350 CFU/mL for the FH strain and 200 CFU/mL for the M129 strain.

e. Assay Reactivity/Inclusivity:

As the primer and probe sequences for the ***illumi****gene* Mycoplasma Direct did not change from the originally cleared assay, a repeat assay reactivity/inclusivity study was not required. It is expected that both Type I and Type II strains will be detected with the Direct assay.

See predicate K123423 for original studies.

f. Analytical Specificity/Cross Reactivity:

A re-evaluation of cross-reactivity with the ***illumi****gene* Mycoplasma Direct DNA amplification assay was not performed. However, an interference study for the Direct assay was completed and demonstrated that the new extraction and DNA processing step included in the SMP PREP II was sufficient and did not result in interference with other substances (both biological and chemical) that could be present in clinical samples. A cross-reactivity study was previously conducted on the predicate device (***illumi****gene* Mycoplasma DNA amplification assay) in submission K123423 for which primers and probes are identical to the Mycoplasma Direct assay. Therefore, because the primer/probe sequences remain unchanged from the predicate and the modified extraction process was successfully clinically evaluated, a cross-reactivity study on the Direct assay was not required. No cross-reactivity is expected with the ***illumi****gene* Mycoplasma Direct assay.

See predicate K123423 for original studies.

g. Interference:

An interference study using 17 potential interfering chemical and biological non-microbial substances was conducted to evaluate the accuracy of results generated by the ***illumi****gene* Mycoplasma Direct DNA amplification assay. Contrived low positive samples P1 (M129) and P2 (FH) were spiked into a simulated negative matrix (Liquid Amies media with screened negative throat gargle and a rayon swab) at 300 CFU/mL and 1762.5 CFU/mL, respectively. Each potential interferent was diluted to specific concentrations in sterile saline and added to one simulated negative sample (N1) and two unique contrived low positive samples (P1, P2). Testing included three replicates for each interferent alone (negative sample) and three replicates for each interferent mixed with each of the two *M. pneumoniae* strains (six positive samples per interferent).

The following substances, at the saturated solvent/diluent concentrations indicated, do not interfere with ***illumi****gene* Mycoplasma Direct assay (no false positive or false negative results):

Substance	**Saturated Solvent/Diluent Concentration**
Acetaminophen	18.1 mg/mL
Albuterol Sulfate	20 mg/mL
Aspirin	9.1 mg/mL
Azithromycin dehydrate	2.0 mg/mL
Cepacol® Mouthwash	[Alcohol (1.4% v/v), Cetylpyridinium chloride (0.005% v/v)]
Contac® Cold + Flu Tablets	[Acetaminophen (14.8 mg/mL), Chlorpheniramine maleate (0.06 mg/mL),
Phenylephrine HCl	0.15 mg/mL
Diphenhydramine HCl	2.6 mg/mL

Erythromycin	20.0 mg/mL
HALLS® Cough Drops	[Menthol (0.06 mg/mL)]
Ibuprofen	12.7 mg/mL
Phenylephrine HCl	0.595 mg/mL
Prednisone	20.0 mg/mL
Robitussin® Cough+Chest Congestion Cough Syrup	[Dextromethorphan HBr (0.20 mg/mL), Guaifenesin (2.0 mg/mL)]
Saline Nasal Spray	[Sodium chloride (0.65 mg/mL)]
Mucus	5.0mg/mL
White Blood Cells	0.5% v/v

All potentially interfering substances that were evaluated yielded acceptable results except whole blood at concentrations > 2% v/v. As with the predicate K123423, phenylephrine HCl (found in nasal decongestants and flu and cold tablets) at concentrations > 0.595 mg/ml interfered with the Mycoplasma Direct assay and caused false negative results with *M. pneumoniae* strain M129. Therefore, this information is provided in the Mycoplasma Direct assay package insert as limitations. During the interference study, Robitussin® Cough+Congestion Cough Syrup initially produced 1/3 invalid results with Positive Sample P2. Repeat testing produced acceptable results (1/1 positive) and therefore, Robitussin® Cough+Congestion Cough Syrup is not considered an interferent. This information is relayed in the package insert.

Assay cut-off:

See predicate K123432 for original studies. Below is a general summary.

There are fixed cut-off values for the ***illumi****gene* Mycoplasma Direct assay that allows sample results to be reported. The pre-selected cut-off values for the TEST and CONTROL chambers of the Mycoplasma Test Device were previously optimized and validated for K123423. Fixed cut-off values for the CONTROL chamber assess validity. CONTROL chamber S_f:S_i ratios less than 90% are considered valid and results from the TEST chamber can be reported (POSITIVE, NEGATIVE, INVALID). CONTROL chamber S_f:S_i ratios greater than or equal to 90% are considered invalid which prevents TEST chamber results from being reported. More stringent cut-off criteria are applied to the CONTROL chamber reaction in order to ensure that amplification is not inhibited, reagents are performing as intended, and 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' and TEST chamber S_f:S_i ratios greater than or equal to 82% are reported as 'NEGATIVE'. Numerical values for the CONTROL and the TEST chamber results are not displayed for the user.

2. Comparison studies:

a. Clinical Sensitivity and Clinical Specificity:

The ***illumi****gene* Mycoplasma Direct DNA amplification assay was evaluated in 2015-2016 by three independent clinical test sites located in the Midwestern, Southern, and Central regions of the United States. A total of 458 throat swab samples were collected from male and female patients suspected of having a *Mycoplasma pneumoniae* infection. The study included testing of prospectively collected throat swabs from pediatric, adult, and geriatric patients that were presumed to be symptomatic with an upper respiratory tract infection caused by *M. pneumoniae*. Double throat swabs were collected per patient; one swab was placed in Liquid Amies transport media and one swab was placed in M4 viral transport media that was only used for discrepant analysis. These clinical throat swab specimens were evaluated with the test device to establish performance characteristics.

The performance of the Mycoplasma Direct assay was compared to a reference molecular in-vitro diagnostic method, the ***illumi****gene* Mycoplasma DNA Amplification Assay that was previously cleared by FDA. ***illumi****gene* Mycoplasma predicate device testing and discrepant testing was performed at Meridian Bioscience and not at the clinical study sites.

Two specimens were excluded from performance analysis due to instrument error and an unacceptable sample type. A total of 456 prospectively collected throat swabs were eligible for analysis. From this clinical study, the overall prevalence of *M. pneumoniae* in the prospective throat swab specimens was 7.5% (34/456). No invalid results were produced by the Mycoplasma Direct assay; however, the reference molecular method yielded 28 invalid results. Repeat predicate testing for these 28 samples yielded results that were identical to the test device and presented in the table below. During clinical evaluation, 11 discordant results were produced. Repeat testing was performed according to the Clinical Trial Protocols and Clinical Trial Discrepant Analysis Plan.

The ***illumi****gene* Mycoplasma Direct assay performance for prospective throat swab specimens as compared to the reference method (***illumi****gene* Mycoplasma) are shown in the tables below. There were no restrictions on age, gender, medications, or known pharmaceutical therapies.

Performance Characteristics of the *illumi*gene Mycoplasma Direct Assay (All Sites Combined)

	***illumi*gene Mycoplasma Predicate Assay**			
***illumigene* Mycoplasma Direct Assay**	**Positive**	**Negative**	**Invalid**	**Totals**
Positive	24	10[a]	0	34
Negative	1[b]	421	0	422
Invalid	0	0	0	0
Totals	25	431	0	456

			95% CI
Overall Percent Agreement	445/456	97.6%	95.7 - 98.6%
Positive Percent Agreement	24/25	96.0%	80.5 - 99.3%
Negative Percent Agreement	421/431	97.7%	95.8 - 98.7%
Invalid Rate	0/456	0%	0.0 - 0.8%

[a] 4/10 samples were identified positive by ***illumi****gene* Mycoplasma after testing with an additional frozen sample.
[b] Repeat testing by ***illumi****gene* Mycoplasma with the original patient sample and an additional frozen sample produced negative results.

Age information was known for all patients included in the performance analysis. Patients ranged in age from 3 weeks to 97 years old.

Performance of the *illumi*gene Mycoplasma Direct Based on Age

Patient Age	Total Positive	Total Samples	Prevalence (%)
0 to 1 Month	0	7	0.0%
2 Months to 2 Years	5	157	3.2%
3 Years to 12 Years	22	147	15.0%
13 Years to 21 Years	5	38	13.2%
22 Years to 65 Years	2	86	2.3%
>65 Years	0	21	0.0%

No performance differences were noted based on chronological age.

The study population included 226 male and 230 female patients. The ***illumi****gene* Mycoplasma Direct detected 18 positives samples from specimens collected from males and 16 positive samples from specimens collected from females. When comparing the ***illumi****gene* Direct to the ***illumi****gene* predicate device, no performance differences were noted based on gender.

Clinical performance of the Direct assay compared to the ***illumi****gene* Mycoplasma predicate assay was also evaluated based on clinical site. From the data presented below, there were no differences in clinical performance between the assays based on clinical site.

Performance of the *illumi*gene Mycoplasma Direct Based on Clinical Site

Clinical Site ID	***illumi****gene* Direct/ ***illumi****gene* Predicate	Positive Percent Agreement	95% CI	***illumi****gene* Direct/ ***illumi****gene* Predicate	Negative Percent Agreement	95% CI
Clinical Site 1	3/4	75.0%	30.1 - 95.4%	147/148	99.3%	96.3 – 99.9%
Clinical Site 2	2/2	100.0%	34.2 -100.0%	45/47	95.7%	85.8 – 98.8%
Clinical Site 3	19/19	100.0%	83.2 - 100.0%	229/236	97.0%	94.0 – 98.6%
All Sites Combined	24/25	96.0%	80.5% - 99.3%	421/431	97.7%	95.8 – 98.7%

b. Matrix comparison:

Sample type equivalency studies were performed to determine the suitability of two non-nutritive transport mediums with three swab types for use with the ***illumi****gene* Mycoplasma Direct DNA amplification assay. The following transport mediums were evaluated: Liquid Amies (without charcoal) and Liquid Stuart. The following swab types were evaluated: rayon, polyester, and flocked nylon.

Simulated negative matrix was prepared by inoculation of Liquid Amies or Liquid Stuart with screened negative throat gargle and one of the three abovementioned swabs. Contrived low positive samples were prepared by inoculating simulated negative matrix with *Mycoplasma pneumoniae* strain M129 to approximately 300 CFU/mL. All negative and positive samples were tested in triplicate for all possible combinations of swab and media type. All negative samples yielded expected results. One contrived positive sample containing a Liquid Stuart/flocked nylon swab combination yielded a negative result. Testing 20 additional contrived positive samples in Liquid Stuart media with flocked nylon swabs yielded no discordant results further demonstrating that media/swab combination can be used with the Mycoplasma Direct assay.

3. Clinical studies: See Comparison Studies under Section M 2 on pages 11-12.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   The overall incidence of *Mycoplasma pneumoniae* in prospectively collected throat swab specimens in the 2015-2016 clinical study was 7.5% (34/456).

N. Instrument Name

illumi*pro-10*™ Automated Isothermal Amplification and Detection System

O. System Description

1. Modes of Operation:

The following principles of operation enable the ***illumi****gene* Mycoplasma Direct assay to detect the presence of *M. pneumoniae* in throat swab specimens:

A *Mycoplasma pneumoniae* throat swab is collected from a patient following appropriate institutional procedures. The swabs are placed in either non-nutritive Liquid Amies (without charcoal) transport media or Liquid Stuart and delivered to the clinical laboratory for testing. The collected throat swab is added directly to the Sample Preparation Apparatus II/Negative Control III reagent that initiates specimen and internal control processing and DNA extraction. The sample/control mixture is passed through a filter on the SMP PREP II and

into heat-treatment tubes to prepare the DNA for amplification and detection. The heat-treated sample/control mixture is added to an ***illumi****gene* Mycoplasma Test Device that contains the TEST and CONTROL chambers.

The Mycoplasma Test Device containing prepared samples and control reagent is placed in the ***illumi****pro-10*™ instrument (incubator/reader). Each test device is heated, facilitating amplification of target DNA. When *M. pneumoniae* is present in a throat swab specimen, a conserved *M. pneumoniae* DNA sequence is amplified and magnesium pyrophosphate is generated as a byproduct of the reaction. Magnesium pyrophosphate forms a precipitate in the reaction mixture causing the solution to become turbid. The ***illumi****pro-10*™ instrument detects the change in light transmission through the reaction mixture created by precipitating magnesium pyrophosphate. The ***illumi****pro-10*™ calculates the ratio between the initial and final absorbance values (S_i:S_f) and compares this ratio to an established cut-off value. Sample results are reported as POSITIVE, NEGATIVE, or INVALID based on a comparison between the calculated absorbance ratio and the fixed cut-off.

2. Software:

The ***illumi****gene* Mycoplasma Direct assay utilizes the same software as the previously cleared predicate device, the ***illumi****gene* Mycoplasma Assay. Since there have been no changes to the internal assay cut-off or other software specifications, the original assay file used in the cleared device is used in the Direct assay. FDA has previously reviewed the applicant's Hazard Analysis and software development processes in K123423.

3. Specimen Identification:

Specimens are identified by position in the heat block wells of the ***illumi****pro-10* instrument. Default Sample Identification is based on Block and Well position (e.g., Block A, Well 1). The user can provide Sample Identification information using the keypad, ***illumi****pro-10* barcode scanner, or the optional external keyboard. Sample information is entered by following instructions displayed on the ***illumi****pro-10* screen.

4. Specimen Sampling and Handling:

Clinical specimens are prepared using the Sample Preparation Apparatus II/Negative Control III reagent included with the ***illumi****gene* Mycoplasma Direct DNA amplification assay. Incubation, loop-mediated isothermal amplification, and detection are automated using the ***illumi****pro*-10™ instrument.

5. Calibration:

Calibration of the ***illumi***pro-10™ is not required. However, verification of a properly functioning OPTICS SYSTEM must be performed monthly for both Block A and B. Stepwise instructions for Optics System Verification are provided on the ***illumi****pro-10* display, utilizing the SERVICE MODE menu. When the ***illumi****pro-10* is powered on, the instrument automatically conducts an internal **Power-On Self Test (POST)** to ensure that

software and hardware components are performing as expected. A POST Test failure is signaled by an Error Code that can be addressed following instructions in the ***illumi****pro-10* Operator's Manual.

6. Quality Control:

The ***illumi****gene* Mycoplasma Direct External Positive Control Kit contains only a positive control reagent and is not a component of the Mycoplasma Direct test system. The External Positive Control is processed using the same DNA extraction methodology as the clinical patient specimens. The ***illumi****gene* Sample Preparation Apparatus II/Negative Control III reagent is built into the ***illumi****gene* Mycoplasma Direct kit and serves as the External Negative Control. In general, External Controls are intended 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. Therefore, the ***illumi****gene* Mycoplasma External Controls are recommended for routine Quality Control. The performance of External Positive and Negative Controls were validated as part of the clinical study.

Each test device also includes an internal control chamber that houses *S. aureus* control DNA. The internal control monitors amplification inhibition, assay reagent performance, and sample processing effectiveness. If the internal control fails, an invalid result is generated and no read-out is given for the TEST chamber of the device.

P. Proposed Labeling:

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

Q. Conclusion:

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