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

A. 510(k) Number:

k123423

B. Purpose for Submission:

To obtain substantial equivalence determination for the ***illumi****gene*® Mycoplasma DNA Amplification Assay

C. Measurand:

Specific DNA sequence from *Mycoplasma pneumoniae*

D. Type of Test:

Molecular assay based on loop-mediated isothermal amplification technology (LAMP)

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

illumi*gene*® Mycoplasma DNA Amplification Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980 – Respiratory Viral Panel Multiplex Nucleic Acid Assay

   21 CFR 862.2570 – Instrumentation for Clinical Multiplex Test Systems

2. Classification:

   Class II

3. Product code:

   OZX – Mycoplasma pneumoniae DNA Assay System

OOI – Real Time Nucleic Acid Amplification System

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   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* 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 infection 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.

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*®

Mycoplasma DNA Amplification Assay Test Kit, the ***illumi****gene*® Mycoplasma External Control Kit and the ***illumi****pro-10*™ Automated Isothermal Amplification and Detection System.

The ***illumi****gene* Mycoplasma assay utilizes loop-mediated isothermal amplification (LAMP) technology to detect the presence of *Mycoplasma pneumoniae* in human respiratory specimens (throat and nasopharyngeal swab specimens). Each ***illumi****gene* Mycoplasma assay is completed using an ***illumi****gene* Assay Control Reagent containing Control material, an ***illumi****gene* Reaction Buffer, an ***illumi****gene* Mycoplasma Test Device and microcentrifuge tubes. Respiratory specimens are combined with the ***illumi****gene* Assay Control Reagent. The Specimen/Control sample is manually extracted and purified using a commercially available extraction kit (Qiagen, QIAamp® DSP DNA Mini Kit). Extracted DNA is heat-treated. Target and Control DNA are made available for isothermal amplification via heat- treatment. The heat-treated Specimen/Control sample is added to the ***illumi****gene* Reaction Buffer. DNA amplification occurs in the ***illumi****gene* Test Device.

The ***illumi****pro-10*™ heats each ***illumi****gene* Mycoplasma Test Device containing prepared sample and control material, facilitating amplification of target DNA. When *M. pneumoniae* is present in the specimen, a 208 base pair sequence of the *M. pneumoniae* genome 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 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 for both the TEST chamber and the CONTROL chamber.

The ***illumi****gene* Mycoplasma External Controls Kit contains a Positive and Negative Control Reagent. 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. External Control reagents are provided for use in routine Quality Control testing.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FilmArray® Respiratory Panel (RP) System

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

   K120267

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Qualitative test for the direct detection and identification of *Mycoplasma pneumoniae* in nasopharyngeal swabs and throat swabs	Qualitative test for the direct detection and identification of *Mycoplasma pneumoniae* (and other organisms) in nasopharyngeal swabs
Organism Detection	*Mycoplasma pneumoniae*	Same
Detection	Self contained and automated	Self contained and automated
Assay Target	DNA	DNA

Differences		
Item	Device	Predicate
Specimen Type	Nasopharyngeal swabs, throat swabs	Nasopharyngeal swabs
Assay Platform	***Illumi****pro-10*™ Automated isothermal Amplification and Detection System	FilmArray Instrument
Amplification Technology and Target Sequence Detected	Singleplex DNA Amplification Assay; Loop-Mediated Isothermal Amplification (LAMP) for the detection of a 208 base pair sequence of the Mycoplasma genome. The ***illumi****pro-10*™ detects changes in reaction solution absorbance by visible light transmission	Multiplex Nucleic Acid Amplification Assay; Assay utilizes freeze-dried reagents to perform nucleic acid purification, reverse transcription, and nested multiplex PCR with DNA melt analysis
Extraction	Separate manual extraction method using Qiagen reagents	Automated extraction included in FilmArray RP pouch
External Assay Controls	External Positive and Negative Controls provided in ***illumi***gene Mycoplasma External Control Kit	External controls not provided
Reading Method	Visible Light Transmission	Fluorescence Emissions
Interpretation of Results	Results of the ***illumi****gene* Mycoplasma Assay are interpreted by the *illumi*pro-10™ and reported as INVALID, POSITIVE and NEGATIVE based on change in light transmission of the reaction mixtures. EMPTY WELL is reported when an ***illumi***gene Test	Results of the FilmArray Respiratory Panel (RP) Assay report are interpreted by the FilmArray Instrument for *M. pneumoniae* and reported as Detected, Not Detected or Invalid.

Differences		
Item	Device	Predicate
	Device is not detected by the ***illumi****pro*-10™ or when questionable Signal Initial (S_i) transmission is detected.	

K. Standard/Guidance Document Referenced:

- Clinical and Laboratory Standards Institute. 2008. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline Second Edition (EP12A)
- Clinical and Laboratory Standards Institute. 2005. User Verification of Performance for Precision and Trueness; Approved Guideline Second Edition (EP15A2)
- Clinical and Laboratory Standards Institute. 2005. Interference Testing in Clinical Chemistry; Approved Guideline Second Edition (EP7A2)

L. Test Principle:

The ***illumi****gene* Mycoplasma 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. Magnesium pyrophosphate is produced as a by product of LAMP amplification. The magnesium pyrophosphate forms a white precipitate in the reaction solution, giving the reaction solution a turbid appearance. Change in sample absorbance created by precipitation of magnesium pyrophosphate indicates the presence of target DNA and is considered a positive reaction. The absence of target DNA results in no detectable change in sample absorbance and is considered a negative reaction.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility Study:

   Blind coded panels of 10 samples were supplied to three independent laboratories. Samples were randomly sorted within each panel to mask sample identities. Each panel consisted of true negative (1 replicate), high negative (three replicates), low positive (three replicates), and moderate positive (three replicates) concentrations. Panel members were prepared in natural negative matrix in M4 non-nutritive transport medium (polyester swab inoculated with normal nasal flora, screened negative for *M. pneumoniae*) and were spiked with *M. pneumoniae* strain M129 to the final concentrations indicated in the table below. The low positive sample concentration was set near the Limit of Detection (LoD) which should generate

positive results ≥ 95% of the time and the high negative sample was set at a concentration that should generate negative results ≥ 95% of the time.

Sample ID	Final Concentration (CFU/Test)	Final Concentration (CFU/mL)	Expected Result
Moderate Positive	30	800	Positive
Low Positive	15	400	Positive
High Negative	0.03	0.8	Negative
Negative	N/A	N//A	Negative

Three lots of ***illumi****gene* Mycoplasma reagents and five ***illumi****pro-10*™ instruments were used in reproducibility studies. Each clinical site tested two panels each day for five days 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:

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	Site 1		Site 2		Site 3		Total				
Sample Type	Percent agreement		Percent agreement		Percent agreement		Percent agreement		Average S_i:S_f	SD	%CV
Negative	10/10	100%	10/10	100%	10/10	100%	30/30	100%	100.4	2.85	2.83%
High Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%	101.1	3.32	3.29%
Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	61.7	6.18	10.02%
Moderate Positive	29/30	96.7%	30/30	100%	29/30	96.7%	88/90	97.8%	62.3	6.25	10.04%
Negative Control	10/10	100%	10/10	100%	10/10	100%	30/30	100%	101.5	4.03	3.97%
Positive Control	10/10	100%	10/10	100%	10/10	100%	30/30	100%	60.5	4.29	7.10%

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 assay. Validation studies performed at Meridian were completed using Rayon and polyester swabs with M4, M4RT, M5, 0.85% Saline, UTMRT, and Liquid Stuart Transport Medium. Samples were prepared in both simulated nasal and throat matrices spiked with the FH strain of *M. pneumoniae* at high negative and low

positive (2x LoD) concentrations.

Testing was performed in triplicate for each storage condition; (1) upper limit of room temperature (32.5ºC) and (2) refrigerated (2-8ºC). All samples tested yielded the correct results for sample storage of up to 49 hours for elevated room temperature storage and up to 6 days at 2-8ºC. Study results demonstrated that throat and nasopharyngeal swabs (both rayon and polyester) in the above described transport media can be held at the claimed storage conditions: 19-29ºC for up to 48 hours or at 2-8ºC for up to 5 days prior to testing.

Extracted Specimen Stability:

Studies were performed to evaluate storage parameters for storage of specimen extracts prior to heat treatment. Samples consisting of rayon and polyester swabs with M5 media in a simulated negative matrix were spiked with *M. pneumoniae* at high negative, LoD and 5x LoD concentrations. Contrived negative samples were also tested. Extracted samples were held at the upper limit of room temperature (29ºC) prior to heat treatment and testing on the ***illumi****pro-10*™. All extracted samples yielded the expected results for storage up to 75 minutes. Study results validated the claimed stability of 19-29ºC for up to 1 hour prior to heat treatment for specimen extracts.

Controls

External Controls: The ***illumi****gene* Mycoplasma External Control Kit includes external positive and negative controls and is sold separately from the ***illumi****gene* Mycoplasma DNA Amplification Assay Kit. The positive external control contains non-infectious plasmid DNA with *M. pneumoniae* and *S. aureus* inserts. The negative external control consists of non-infectious plasmid DNA with a *S. aureus* insert. 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 kits. External control testing should be performed thereafter in accordance with appropriate federal, state and local guidelines. Results from the ***illumi****gene* Mycoplasma test kit should not be reported if external controls do not produce the expected results.

Internal Control: Primers for amplification of the internal control are incorporated into each device in the CONTROL chamber. Internal control DNA (Assay Control II Reagent- phosphate-buffered solution containing formalin-treated *E. coli* harboring plasmid containing a segment of the *Staphylococcus aureus* genome) is combined with each specimen and processed through all steps of the procedure. The internal control is present to monitor for sample processing, amplification inhibition, and assay reagent integrity.

d. Detection limit:

Analytical Sensitivity studies were designed to determine the analytical limit of detection (LoD) of the ***illumi****gene* Mycoplasma 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%). Two *M. pneumoniae* strains, FH (ATCC 15531) and M129, were evaluated. Aliquots from the two strains were sent to an independent testing facility for concentration determination by colony count. Samples were prepared in a natural negative matrix (rayon swabs in M4 non-nutritive Transport Medium inoculated with normal nasal flora previously screened negative for *M. pneumoniae*). Replicates for each dilution were tested with the ***illumi****gene* Mycoplasma 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 LoD. Testing was performed using three lots of reagents and six ***illumi****pro-10*™ instruments. External Positive and Negative Controls were tested each day throughout the study. The LoD for the assay was confirmed as 88 CFU/Test (2,350 CFU/mL) for the FH strain (ATCC 15531) and 7.5 CFU/Test (200 CFU/mL) for the M129 strain.

e. Analytical Reactivity/Inclusivity:

Nine additional strains (see table below) of *M. pneumoniae* were tested with the ***illumi****gene* Mycoplasma DNA Amplification Assay and produced the expected positive results at or below stated assay LoD of 88 CFU/Test (2,350 CFU/mL). Samples were diluted in sterile saline and spiked into negative matrix which consisted of rayon swabs inoculated with normal nasal flora and placed into M4 Transport Media. The nasal matrix was previously screened as negative for *M. pneumoniae*. Three replicates of each strain were tested resulting in 100% detection. Both *M. pneumoniae* Type 1 and Type 2 strains were represented.

Strain Identification	**Strain Identification**
PI1428	Mutant 22 (ATCC 39505)
MAC (ATCC 15492)	UAB 55612
M52(ATCC 15293)	UAB 56317
Bru (ATCC 15377)	UMTB-10G (ATCC 49899) *M. pneumoniae* and *M. genitalium*)
M129-B170 (ATCC 29343)	

f. Interference:

An interference study was carried out to evaluate the effect of potentially interfering substances on the accuracy of test results obtained with the ***illumi****gene* Mycoplasma DNA Amplification Assay. Potentially interfering substances were diluted in sterile saline and added to M4 medium with rayon swabs (negative sample) and to M4 medium with polyester swabs (positive samples). Two sets of positive samples were spiked separately with *M. pneumoniae* strain M129 and with *M. pneumoniae* strain FH (ATCC 15531) at

concentrations near the LoD. 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).

Testing of the following biological substances at the concentrations indicated demonstrated no interference (no false positive or false negative results): Mucus (5.0 mg/mL), White blood cells (0.5% v/v), and whole blood (5% V/V).

Testing of the following chemical substances at the concentrations indicated demonstrated no interference (no false positive or false negative results): Acetaminophen (18.1 mg/mL), Albuterol Sulfate (20 mg/mL), Aspirin (9.1 mg/mL), Azithromycin dehydrate (2.0 mg/mL), Cepacol® Mouthwash [Ethanol, denatured (1.4% v/v), Cetylpyridinium chloride (.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 [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)].

The presence of phenylephrine HCl (found in nasal decongestants and flu and cold tablets) at concentrations above 0.595 mg/mL yielded false negative results with *M. pneumoniae* strain M129. This information is included as a limitation in the package insert.

g. Analytical specificity/Cross-reactivity:

Potentially cross-reacting microorganisms expected to be present in throat or nasopharyngeal swab specimens were added to samples both negative and positive for *M. pneumoniae*. Negative samples were prepared with M4 transport medium inoculated with nasal flora on rayon swabs and did not contain *M. pneumoniae*. Positive samples were prepared by spiking confirmed negative sample matrix (M4 transport medium inoculated with nasal flora on polyester swabs) with *Mycoplasma pneumoniae,* FH strain (ATCC 15531) at concentrations near the LoD. Three replicates were tested for both positive and negative samples and for each potential cross-reacting organism.

Samples of each potentially cross-reactive (or interfering) microorganism were prepared at minimum concentrations of $\geq 1.0 \times 10^6$ CFU/mL for bacteria/fungi and $\geq 1.0 \times 10^5$ $TCID_{50}$/mL for viruses. Human DNA was tested at 2.0 ng/test.

None of the following organisms demonstrated interference or cross-reactivity with the ***illumi****gene* Mycoplasma DNA Amplification Assay: *Acinetobacter baumannii*, *Acinetobacter calcoaceticus, Actinomyces odontolyticus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Bordetella parapertussis*, *Bordetella pertussis*, *Burkholderia cepacia*, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Chlamydia pneumoniae*, *Citrobacter freundii*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*,

Escherichia coli (ESBL), *Fusobacterium nucleatum*, *Haemophilus ducreyi*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Klebsiella pneumoniae* (KPC), *Legionella pneumophila*, *Listeria monocytogenes*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Neisseria cinerea*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Peptostreptococcus anaerobius*, Proteus *mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* (Group A), *Salmonella typhimurium* (Group B), *Serratia liquefaciens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* (Group B), *Streptococcus anginosus* (Group F), *Streptococcus bovis* (Group D), *Streptococcus canis* (Group G), *Streptococcus equisimilis*, *Streptococcus mitis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus salivarius*, *Ureaplasma urealyticum*, Adenovirus, Coxsackievirus, Cytomegalovirus, Epstein Barr virus, Herpes simplex virus 1, Herpes simplex virus 2, Influenza A, Influenza B, Human metapneumovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Respiratory syncytial virus A, Respiratory syncytial virus B, Rhinovirus, Human DNA.

Moraxella catarrhalis, *Nocardia asteroides*, and Coronavirus each produced false negative results in one of three *M. pneumoniae*-positive replicates tested. Testing of an additional four replicates for each of the three potentially interfering organisms was performed with all additional replicates yielding the expected positive results. Therefore for each of the three organisms, false-negative results occurred in one of seven replicates.

h. Assay cut-off:

The ***illumi****gene* Mycoplasma is manufactured with fixed cut-off values. The product is designed around 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 are 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 Sf:Si ratios less than 90% are considered valid and allow for reporting of TEST chamber results (POSITIVE, NEGATIVE). CONTROL chamber Sf:Si ratios greater than or equal to 90% are considered invalid. Test chamber results are not reported for samples with an "INVALID" control result. 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 Sf:Si ratios less than 82% are reported as 'POSITIVE' and TEST chamber Sf:Si 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.

i. Fresh/Frozen Studies:

Meridian performed a fresh versus frozen study with 60 samples contrived from 30 fresh nasal wash specimens determined to be negative for *M. pneumoniae* using a validated PCR assay. Each nasal wash specimen was tested both with and without *M. pneumoniae* spiked into the sample. Spiked specimens were prepared at concentrations near the LoD (7.5 CFU/test or 200 CFU/mL, initially tested with the **illumi**gene assay, and tested again after each of three freeze/thaw cycles. Study results demonstrated 100% agreement for all negative samples for each freeze/thaw cycle. For positive specimens, 29/30 specimens demonstrated the expected positive result. One positive sample gave a false negative result after the first freeze/thaw cycle but this same sample was positive after the second and third freeze/thaw cycles, indicating that the sample had not deteriorated. Results of the study sufficiently demonstrated that fresh and frozen specimens should provide equivalent results with the ***illumi****gene* Mycoplasma DNA Amplification Assay.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable, performance of the assay was evaluated in comparison to a composite reference method of culture and PCR followed by bi-directional sequencing.

b. Matrix comparison:

Sample Type Equivalence Study:

Sample type equivalency studies were performed to confirm the suitability of several swab types as well as several non-nutritive transport media for use with the ***illumi****gene* Mycoplasma DNA Amplification Assay. The following swab types were evaluated: Cotton, Flocked Nylon, Foam, Polyester, and Rayon. The following non-nutritive transport mediums were evaluated: M4, M4-RT, M5, Liquid Stuart, 0.85% Saline and UTM-RT (Universal Transport Medium).

Contrived positive samples were prepared by combining confirmed *M. pneumoniae*-negative matrices with the FH strain of *M. pneumoniae* at concentrations near the LoD (2670 CFU/mL for NP swabs, and 920 CFU/mL for throat swabs. Leftover nasal wash and throat specimens were used to prepare specimens for the study. Replicates of both positive and negative samples were tested for all possible combinations of specimen matrix, swab, and media type. All negative samples yielded correct results.

Three positive samples gave unexpected negative results: One replicate of a NP matrix/rayon swab/M5 media combination and two replicates of a throat matrix/polyester swab/M4 media combination.

Testing of four additional NP/rayon/M5 media samples yielded no discordant results. Additionally, each of three variables (NP, rayon, M5) was challenged in combination with other matrices, swab types and media types resulting in no additional false negative results. Therefore, it is likely that the one original discordant result was likely due to the use of *M. pneumoniae* concentrations near the LoD.

Twenty additional samples prepared in throat matrix/polyester swab/M4 media were tested in a simulated matrix equivalency study with all 20 samples yielding expected positive results. Therefore, it is likely that the two original discordant results for this swab/media combination were likely due to the use of *M. pneumoniae* concentrations near the LoD.

Swab and transport medium types with demonstrated performance are described in the ***illumi****gene* Mycoplasma DNA Amplification Assay Package Insert.

3. Clinical Studies:

a. Clinical Sensitivity and specificity

The ***illumi****gene* Mycoplasma DNA Amplification Assay was evaluated in 2012 by five independent clinical test sites located in the Midwestern, Southern, and Central regions of the United States. A total of 334 throat and nasopharyngeal (NP) swab specimens, collected from patients suspected of *Mycoplasma pneumoniae* infection were evaluated with the test device to establish performance characteristics.

The study included testing of leftover de-identified specimens submitted to the testing laboratories for routine *M. pneumoniae* testing. Specimens were presumed to be from symptomatic patients. Testing included prospectively collected and tested fresh specimens, prospectively collected and retrospectively tested frozen specimens (all comers), and retrospectively tested frozen selected specimens.

The performance of ***illumi****gene* Mycoplasma DNA Amplification Assay was compared to a composite reference method that included *M. pneumoniae* culture and identification and a validated real-time PCR assay followed by bi-directional sequencing for positive specimens. The culture portion of the composite reference method was performed at four of the testing sites with one testing site sending specimens to a reference laboratory for culture. All reference PCR assays were performed by Meridian BioScience, Inc with sequencing performed by a reference laboratory for positive specimens. For performance calculations, specimens positive by culture or positive by real-time PCR and bi-directional sequencing were considered positive. Specimens negative by both culture and PCR were considered negative.

A total of 12 specimens were excluded from the performance analysis due to inconclusive culture results and negative PCR results. A total of 103 (30.8%)

prospective and 219 retrospective specimens (65.6%) were tested with one initial invalid result (0.30%). The ***illumi****gene* assay performance for both prospective and retrospective nasopharyngeal and throat swab specimens as compared to the composite reference method are shown in the tables below. Also shown is the ***illumi****gene* assay performance as compared to the reference PCR and bi-directional sequence testing.

illumi*gene* **Mycoplasma Assay Performance: Nasopharyngeal Swabs**

Specimen Description	Positive Specimens			Negative Specimens			Invalid Results
	illumigene **vs. Comparator**	**% Sensitivity or PPA**	**95% CI**	***illumigene*** **vs. Comparator**	**% Specificity or NPA**	**95% CI**	
Composite Method Comparator							
Prospective	4/4	% Sensitivity 100%	51.0 – 100.0%	48/48	% Specificity 100%	92.6 – 100.0%	0
Retrospective	34/36	PPA 94.4%	81.9 – 98.5%	86/90	NPA 95.6%	89.1 – 98.3%	0
Validated PCR Assay with Bi-directional Sequencing Comparator							
Prospective	4/4	% Sensitivity 100%	51.0 – 100.0%	48/48	% Specificity 100%	92.6 – 100.0%	0
Retrospective	34/36	PPA 94.4%	81.9 – 98.5%	86/90	NPA 95.6%	89.1 – 98.3%	0

illumi*gene* **Mycoplasma Assay Performance: Throat Swabs**

Specimen Description	Positive Specimens			Negative Specimens			Invalid Results
	illumigene **vs. Comparator**	**% Sensitivity or PPA**	**95% CI**	***illumigene*** **vs. Comparator**	**% Specificity or NPA**	**95% CI**	
Composite Method Comparator							
Prospective	8/8	% Sensitivity 100%	67.6 – 100.0%	43/43	% Specificity 100%	91.8 – 100.0%	0
Retrospective	22/26[a]	PPA 84.6%	66.5 – 93.9%	66/67	NPA 98.5%	92.0 – 99.7%	1

Validated PCR Assay with Bi-directional Sequencing Comparator							
Prospective	8/8	% Sensitivity 100%	67.6 – 100.0%	43/43	% Specificity 100%	91.8 – 100.0%	0
Retrospective	21/21[b]	PPA 100%	84.5 – 100.0%	70/72	NPA 97.2%	90.4 – 99.2%	1

a. Four specimens originally identified by culture as positive were not confirmed by either the ***illumi****gene* assay or the independent PCR method. Results suggest sample degradation during storage.

b. One specimen originally identified by the ***illumi****gene* assay and culture as positive was negative by the independent PCR method. This specimen is classified as a false positive relative to PCR.

Age information was known for 83.5% (269/322) of patients included in the performance analysis. Seven (2.6%) patients tested were between 0-28 days of age; 38 (14.1%) patients were between 29 days and up to 2 years of age; 139 (51.7%) patients were between 2 and up to 12 years of age; 61 (22.7%) patients were between12-18 years, and 9(3.3%) patients were between 18-21 years of age. The remaining 15 (5.6%) study patients were 21 years or older. No performance differences were noted based on chronological age.

The study population included 90 (27.9%) female patients and 91 (28.3%) male patients. Gender was unknown for 141 (43.8%) of the study participants. No performance differences were noted based on gender.

Clinical performance of the ***illumi****gene* Mycoplasma DNA Amplification Assay was assessed by testing de-identified nasopharyngeal and throat swab specimens lacking clinical information; accordingly, the number of patients with *M. pneumoniae* pneumonia included in the clinical studies was unknown and performance for this group cannot be described separately.

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Overall incidence of *Mycoplasma pneumoniae* in prospectively collected and tested specimens during the 2012 clinical study was 11.7% (12/103).

N. Instrument Name:

illumi*pro-10*™

O. System Descriptions:

1. Modes of Operation:

The ***illumi****pro-10*™ instrument heats each ***illumi****gene* Mycoplasma Test Device containing prepared samples and Control Reagent, facilitating amplification of target DNA. When *M. pneumoniae* is present in a nasopharyngeal or throat swab specimen, a conserved *M. pneumoniae* DNA sequence is amplified and magnesium pyrophosphate is generated. Magnesium pyrophosphate forms a precipitate in the reaction mixture. 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.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ____x____ or No _____

3. Specimen Identification:

   Specimens are identified by position. Default Sample Identification is based on Block and Well position (e.g., Block A, Well 1). The user may input Sample Identification information using the keypad or the optional external keyboard.

4. Specimen Sampling and Handling:

   Specimens are prepared manually. Incubation, amplification and detection are automated using the ***illumi****pro*-10™.

5. Calibration:

   Calibration of the ***illumi***pro-10™ is not required.

6. Quality Control:

   The ***illumi****gene* Mycoplasma External Control Kit contains a Positive and a Negative Control Reagent. The External Positive and Negative Controls are not built into the test system, but are tested in the same manner as a patient specimen. The ***illumi****gene* Mycoplasma External Control Kit is 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. The ***illumi****gene* Mycoplasma External Controls are recommended for routine Quality Control.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

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

R. Conclusion:

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