

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

**A. 510(k) Number:**

K152285

**B. Purpose for Submission:**

To make three modifications to the upfront sample preparation of the original K133673 *illumigene* Pertussis DNA Amplification Assay performed on the *illumipro-10*<sup>TM</sup>. The modifications are:

1. Modify sample preparation to retain the specimen swab in the Sample Buffer Tube at the end of the elution step.
2. Add the Copan Liquid Amies Elution Swab (ESwab<sup>TM</sup>) Collection and Transport System (Copan Diagnostics, K061301) as an acceptable sample type.
3. Add an optional sample pretreatment method to neutralize the interfering activity of biological substances found in the nasopharynx of some patients.

**C. Measurand:**

DNA target sequence: IS481 Insertion Sequence

**D. Type of Test:**

The *illumigene* Pertussis DNA Amplification Assay is a qualitative *in vitro* diagnostic device for the direct detection of *Bordetella pertussis* DNA in human nasopharyngeal swab (NP) samples. The assay uses isothermal loop-mediated DNA amplification (LAMP) technology that targets the IS481 insertional element of the *Bordetella pertussis* genome.

**E. Applicant:**

Meridian Bioscience Inc.

**F. Proprietary and Established Names:**

*illumigene*<sup>®</sup> Pertussis DNA Amplification Assay

**G. Regulatory Information:**

1. Regulation section:

866.3980: Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:

II

3. Product code:

OZZ, *Bordetella pertussis* DNA Assay System

4. Panel:

Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The *illumigene* Pertussis DNA Amplification Assay, performed on the *illumipro-10*<sup>TM</sup>, is a qualitative *in vitro* diagnostic test for the direct detection of *Bordetella pertussis* in human nasopharyngeal swab samples taken from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis*.

The *illumigene* Pertussis assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect *B. pertussis* by targeting the IS481 insertional element of the *B. pertussis* genome. The IS481 insertional element can also be found in *B. holmesii* and *B. bronchiseptica* strains. Respiratory infection with *B. pertussis*, *B. holmesii* or *B. bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis*. *B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

Negative results for the *illumigene* Pertussis DNA Amplification Assay do not preclude *Bordetella pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the *illumigene* Pertussis assay should be used in conjunction with information obtained during the patient's clinical evaluation as an aid in diagnosis of *B. pertussis* infection and should not be used as the sole basis for treatment or other patient management decisions.

*illumigene* Pertussis 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:

*illumipro-10*<sup>TM</sup> Automated Isothermal Amplification and Detection System

**I. Device Description:**

The *illumigene* Molecular Diagnostic Test System is comprised of the *illumigene* Pertussis DNA Amplification Assay Test Kit, the *illumigene* Pertussis External Control Kit and the *illumipro-10*<sup>TM</sup> Automated Isothermal Amplification and Detection System. The *illumigene* Pertussis assay utilizes loop-mediated isothermal amplification (LAMP) technology to detect the presence of *Bordetella pertussis* in human nasopharyngeal swab specimens. The assay targets a 198 base pair sequence of the *Bordetella pertussis* genome residing in a region of the IS481 insertional element sequence. Each *illumigene* Pertussis assay is completed using an *illumigene* Assay Control/Negative Control Reagent containing Control material, an *illumigene* Sample Buffer tube, an *illumigene* Pertussis Test Device and Mineral Oil.

Nasopharyngeal swab specimens are eluted with *illumigene* Sample Buffer by inserting and retaining the swab in the Sample Buffer Tube followed by vortexing. In the case of the ESwab System the collection tube is simply vortexed prior to the next steps. An optional pretreatment/treatment step can also be performed to eliminate inhibitory activity of biological substances found in a patient's sample by addition of the Pretreatment Reagent. Eluted sample, from the Sample Buffer Tube or the ESwab System collection tube is added to an Assay/Control/Negative Control Tube, vortexed and heated at 95°C for 10 minutes. The heat-treated Specimen/Control sample is added to the *illumigene* Test Device. Mineral oil is added to the *illumigene* Test Device to prevent evaporation. DNA amplification occurs in the *illumigene* Test Device. ESwab<sup>TM</sup> comprises of 1 mL of Liquid Amies and a FLOQSwab<sup>TM</sup>. The volume of ESwab medium utilized for testing is 25 µL as it delivers the same number of organisms as does a rayon swab in transport medium (worst-case swab type).

The *illumipro-10*<sup>TM</sup> heats each *illumigene* Pertussis Test Device containing prepared Sample and Control material, facilitating amplification of target DNA. When *B. pertussis* is present in the specimen, target DNA is amplified and magnesium pyrophosphate is generated, forming a precipitate in the reaction mixture.

The *illumipro-10*<sup>TM</sup> monitors the absorbance characteristics of the reaction solutions at the assay Run Start (Signal<sub>initial</sub>, S<sub>i</sub>) and at the assay Run End (Signal<sub>final</sub>, S<sub>f</sub>). The *illumipro-10*<sup>TM</sup> calculates the ratio of S<sub>f</sub> to S<sub>i</sub> and compares the ratio to an established cut-off value. The *illumipro-10*<sup>TM</sup> software calculates this ratio for both the TEST chamber and the CONTROL

chamber. S<sub>f</sub>:S<sub>i</sub> information obtained from each *illumipro-10*<sup>TM</sup> instrument is not available to the end-user. The *illumipro-10*<sup>TM</sup> instrument reports and stores final results for the end-user as Positive, Negative, or Invalid.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
*illumigene*<sup>®</sup> Pertussis DNA Amplification Assay
2. Predicate 510(k) number(s):  
K133673
3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device <i>illumigene</i><sup>®</sup> Pertussis (K152285)</b>	<b>Predicate <i>illumigene</i><sup>®</sup> Pertussis DNA Amplification Assay (K133673)</b>
Intended Use	Same as predicate	Qualitative detection of a <i>Bordetella pertussis</i> DNA sequence from nasopharyngeal specimens
Detection	Same as predicate	Self-contained and Automated
Analyte	Same as predicate	DNA
Controls	Same as predicate	Internal Control Provided
Organisms Detected	Same as predicate	<i>Bordetella pertussis</i>
External Controls	Same as predicate	External positive control included in <i>illumigene</i> Pertussis External Control Kit
Test Format	Same as predicate	DNA Amplification; Loop-Mediated Isothermal Amplification (LAMP)
Assay Target	Same as predicate	<i>Bordetella pertussis</i> IS481 Insertional Element
Instrumentation	Same as predicate	<i>illumipro-10</i> <sup>TM</sup>
Sample Processing	Same as predicate	Manual extraction (for below for differences)
Reading Method	Same as predicate	Visible Light Transmission

<b>Differences</b>		
<b>Item</b>	<b>Device <i>illumigene</i><sup>®</sup> Pertussis (K152285)</b>	<b>Predicate <i>illumigene</i><sup>®</sup> Pertussis DNA Amplification Assay (K133673)</b>
Sample Processing	Sample preparation retains the specimen swab in the Sample Buffer Tube at the	Specimen swab removed from the Sample Buffer Tube at the end of the elution step.

Differences		
Item	Device <i>illumigene</i> <sup>®</sup> Pertussis (K152285)	Predicate <i>illumigene</i> <sup>®</sup> Pertussis DNA Amplification Assay (K133673)
	end of the elution step.	
Sample Processing	Copan Liquid Amies Elution Swab (ESwab™) Collection and Transport System (Copan Diagnostics, K061301) added as an acceptable sample type.	Not applicable
Sample Processing	Optional sample pretreatment method included to neutralize the interfering activity of biological substances found in the nasopharynx of some patients. Pretreatment Reagent can be added to untested and previously tested patient specimens.	Not applicable

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

None

**L. Test Principle:**

The *illumigene* Pertussis 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:* See predicate K133673 for original studies.

- b. *Linearity/Assay Reportable Range*: See predicate K133673 for original studies.
  - c. *Traceability, Stability, Expected Values (Controls, Calibrators, or Methods)*: See predicate K133673 for original studies.
  - d. *Detection Limit*: See predicate K133673 for original studies. Limit of Detection (LoD) studies were not repeated for the device modifications because the addition of the Pretreatment Reagent is limited to the minor dilution effects of adding the Pretreatment Reagent to 500 µL of nasopharyngeal sample or to the ESwab medium. The calculated difference for the sample input dilution factor between the predicate device and the modified device is 7%. Two validation studies were performed to support that the LoD was not affected by the modifications. Samples for validation studies used to support the proposed modifications to the predicate device (K133673) were spiked close to the LoD of the assay (see Comparison Studies below).
  - e. *Analytical Reactivity*: See predicate K133673 for original studies.
  - f. *Analytical Specificity*: See predicate K133673 for original studies.
  - g. *Microbial Interference*: See predicate K133673 for original studies.
  - h. *Interfering Substances*: See predicate K133673 for original studies.
  - i. *Fresh/Frozen Studies*: See predicate K133673 for original studies.
  - j. *Swab Type and Transport Media Equivalence Study*: See predicate K133673 for original studies.
  - k. *Assay cut-off*: See predicate K133673 for original studies.
  - l. *Carryover Study*: See predicate K133673 for original studies.
2. Comparison studies:

a. *Method comparison with predicate device*:

A validation study was performed to evaluate the effect of retaining the swab head and the addition of the optional Pretreatment Reagent on eluted specimen hold times. Step 1 of the Specimen Preparation section of the predicate package insert allows users to hold eluted specimens “at room temperature (21-30 °C) for up to 48 hours or refrigerated (2-8 °C) for up to 7 days prior to testing” after which the swab head was removed from the Sample Buffer Tube. The retained swab-Pretreatment Reagent combination represents the worst case condition; this condition was assessed in the following hold-time study.

Negative matrix was prepared from donor flocked nasopharyngeal (NP) swabs

(Copan FLOQswab, worst-case swab type). Swabs were screened by *illumigene* Pertussis to confirm their negative status. Contrived negative samples were prepared with the donor flocked nylon NP swabs which were placed in Sample Buffer tubes containing 500  $\mu$ L of buffer and 50  $\mu$ L of Pretreatment Reagent. Contrived positive samples differed from negative contrived samples in that Sample Buffer tubes containing the donor flocked nylon NP swabs were inoculated with quantified *B. pertussis* strain BAA-589 to a final concentration 2 x LoD; which when diluted with Pretreatment Reagent represented concentrations near the LoD.

Once prepared, the contrived positive and negative samples were stored at 2-8 °C and just above the upper limit for room temperature (i.e., 21-30 °C) for defined time periods to assess performance. Tests were performed according to the method described in the subject package insert. The *illumipro-10*<sup>TM</sup> instruments utilized in this study used the software version, 2.00:602, the same version used for the predicate. The maximum time points selected for the study were in excess of the limits for hold times defined in the package insert.

Daily testing of external control reagents yielded the expected results. Eluted specimens containing swab heads and Pretreatment Reagent produced the expected results at (1) time 0 (baseline), (2) at hold times of 25+ and 49+ hours at room temperature, and (3) at hold times of 4 days and 8 days under refrigeration. There were no significant differences in  $S_f:S_i$  ratios of the different time points for the same sample resulting in 100% agreement between positive and negatives to expected positive or negative result. These data are comparable to those previously obtained in hold studies with eluted samples (K133673). The eluted sample hold times (i.e., 48 hours when held at 21-30 °C and 7 days if held at 2-8 °C) can be applied to eluted specimens retaining swab specimens with or without Pretreatment Reagent. No invalids were observed during this study.

Another validation study was performed to evaluate the impact of holding ESwab medium samples containing Pretreatment Reagent (worst case condition) under three testing conditions 1) time 0 (baseline), (2) at hold times of 25+ and 49+ hours at room temperature, and (3) at hold times of 4 days and 8 days under refrigeration. The maximum time points selected for the study were in excess of the limits for hold times defined in the package insert. Nasal wash from negative donors was used as negative matrix. Nasal wash samples were screened by *illumigene* Pertussis to confirm their negative status. Contrived negative samples were then prepared by adding the dry FLOQSwab component of the ESwab system to the tube component of the system that contained 1000  $\mu$ L of ESwab medium (liquid Amies), followed by the addition of 50  $\mu$ L of the negative matrix. Fifty  $\mu$ L of Pretreatment Reagent was added to each contrived specimen. Contrived positive samples differed from negative contrived samples in that the negative matrix added to each ESwab system was inoculated with quantified *B. pertussis* strain BAA-589 to a final concentration 2 x LoD; which when diluted with Pretreatment Reagent represented concentrations near the LoD. Prepared samples were stored at 2-8 °C or just above the upper limit for room temperature (i.e., 21-30 °C) storage for defined time periods.

Daily testing of external control reagents yielded the expected results. ESwab medium specimens containing Pretreatment Reagent produced the expected results at (1) time 0 (baseline), (2) at hold times of 25+ and 49+ hours at room temperature, and (3) at hold times of 4 days and 8 days under refrigeration. There were no significant differences in  $S_f$  to  $S_i$  ratios of the different time points for the same sample resulting in 100% agreement between positive and negatives to expected result. Performance of this sample was not affected by the presence of Pretreatment Reagent. No invalids were observed in the study.

*b. Matrix comparison:*

*1) Matrix Equivalency Study:* See predicate K133673 for original studies.

*2) Sample Buffer (Elution buffer) Equivalence Study:* See predicate K133673 for original studies.

**3. Clinical studies:**

*a. Clinical Sensitivity:* See predicate K133673 for original studies.

*b. Clinical specificity:* See predicate K133673 for original studies.

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

Preliminary design work identified that some healthy, asymptomatic persons carried similar biological inhibitors in their nasopharynx as some symptomatic patients. Specimens from asymptomatic patients were used to establish the optimum pretreatment procedure. Final validation of the method and Pretreatment Reagent was performed using specimens both from specifically selected asymptomatic (Study 1) and symptomatic (Study 2) populations to enrich for specimens containing inhibitory activity.

*Study 1:* Rayon and flocked nylon swabs, previously cleared for use with the predicate (K133673) device, were used to collect specimens from healthy, asymptomatic subjects. All swabs were tested immediately using the predicate testing method with retesting of all invalid results. The same set of specimens were then treated with the Pretreatment Reagent and tested after pretreatment with any invalid result retested. All predicate, invalid and pretreatment testing was completed within a 24 hour period. The results of this study are shown in the Table below.

	Rayon Swabs		Flocked Nylon Swabs	
	Predicate Method	Pre-treatment Method	Predicate Method	Pre-treatment Method
<b>Negative</b>	54	54	44	54
<b>INVALID</b>	0	0	10	0

Invalids were not observed with rayon swab samples. In contrast, invalid results were observed in 10 of 54 flocked nylon swab samples which were resolved by the use of the Pretreatment Reagent. Testing using the Pretreatment Reagent method did not produce any false-positive or invalid test results during this study.

*Study 2:* A multi-site clinical evaluation was conducted from April to June 2015 at four selected test sites with prospectively collected specimens from symptomatic patients. Of the 164 specimens collected for this study, 145 met study criteria. The swabs used in this study were previously cleared for use with the predicate (K133673) device. The majority of specimens submitted to the test sites were collected with rayon tipped swabs; one sample was collected a flocked nylon swab and three samples with a polyester swab. The majority of the specimens were tested on the same day of collection. Collected specimens were tested immediately after collection, first using the predicate testing swab method, with retesting of all invalid results. Following testing with the predicate method the same specimens were then treated with the Pretreatment Reagent and tested after pretreatment with any invalid result retested. All predicate, invalid and pretreatment testing was completed within a 24 hour period. Of the nineteen specimens producing invalid test results by the predicate method, 15 were repeatedly invalid on retesting; three were negative and one positive on retesting. Following addition of Pretreatment Reagent these nineteen specimens, that initially gave invalid results, gave 17 negative and 2 positive results. Six specimens gave a result with the predicate method, but produced an invalid result upon treatment with the Pre-treatment Reagent. The data is presented in the Table below.

	<b>Test Sites Percent Agreement (Samples in Agreement/Total Tested)</b>				
	<b>Site 1</b>	<b>Site 2*</b>	<b>Site 3</b>	<b>Site 4</b>	<b>Total</b>
<b>Percent Positive Agreement (PPA)</b>	87.5% (6/7) (CI: 48.7-97.4%)	0% (0/0)	100% (2/2) (CI: 34.2-100%)	100% (6/6) (CI: 61.8-100%)	93.3% (14/15) (CI: 70.2-98.8%)
<b>Percent Negative Agreement (NPA)</b>	94.3% (50/53) (CI: 84.6-98.1%)	93.3% (14/15) (CI: 70.2-98.8%)	100% (15/15) (CI: 79.6-100%)	100% (26/26) (CI: 87.1-100%)	96.3% (105/109) (CI: 90.9-98.6%)
<b>Initial Invalid Rate without Pretreatment Reagent</b>	1.6% (1/63)	0% (0/16)	37.0% (10/27)	20.5% (8/39)	13.1% (19/145)
<b>Initial invalid rate with Pretreatment Reagent</b>	4.8% (3/63)	6.25% (1/16)	3.7% (1/27)	2.6% (1/39)	4.1% (6/145)

\* Site 2 data represents first pass data only as this site did not retest INVALID untreated specimens, as required by the package insert, and before treating INVALID samples with Pretreatment Reagent. All other sites repeated INVALID test samples before pretreating specimens.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

See predicate K133673

**N. Instrument Name:**

*illumipro-10*<sup>TM</sup> Automated Isothermal Amplification and Detection System

**O. System Descriptions:**

1. Modes of Operation:

See predicate K133673 for original description.

2. Software:

See predicate K133673 for original description.

3. Specimen Identification:

See predicate K133673 for original description.

4. Specimen Sampling and Handling:

Nasopharyngeal swab specimens are eluted with *illumigene* Sample Buffer by inserting and retaining the swab in the Sample Buffer Tube followed by vortexing. In the case of the ESwab System, the collection tube is simply vortexed prior to the next steps. An optional pretreatment/treatment step can also be performed to eliminate inhibitory activity of biological substances found in a patient's specimen by addition of the Pretreatment Reagent. Specimens are processed manually prior to inoculation of test cartridges which are then placed into the *illumipro-10*<sup>TM</sup> instrument for automated amplification and detection.

5. Calibration:

Calibration of the *illumipro-10*<sup>TM</sup> is not required.

6. Quality Control:

See predicate K133673 for original description.

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

None

**Q. Proposed Labeling:**

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

**R. Conclusion:**

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