**PRINCIPLE:**

The ***illumi****gene* Pertussis DNA Amplification Assay, performed on the ***illumi****pro-10™*, 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 ***illumi****gene* Pertussis assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect *Bordetella pertussis* by targeting the IS481 insertional element of the *Bordetella pertussis* genome. The IS481 insertional element can also be found in *Bordetella holmesii* and some *Bordetella bronchiseptica* strains. Respiratory infection with *Bordetella pertussis*, *Bordetella holmesii* or *Bordetella bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*. 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.

Loop-mediated amplification uses specially designed primers to provide for specific and continuous isothermal DNA amplification. A by-product of this amplification is the formation of magnesium pyrophosphate, which forms a white precipitate leading to a turbid reaction solution. Changes in reaction solution absorbance characteristics created by precipitation of magnesium pyrophosphate indicate the presence of target DNA. The absence of target DNA results in no significant change in sample absorbance.

The ***illumi****gene* Pertussis kit includes ***illumi****gene* Pertussis Assay Control/Negative Control Reagent, ***illumi****gene* Pertussis Test Devices, ***illumi****gene* Pertussis Sample Buffer and Mineral Oil. The ***illumi****gene* Assay Control/Negative Control, used for specimen dilution and preparation, is a Tris-buffered solution containing formalin-treated *E. coli* harboring *Staphylococcus aureus* DNA. The *S. aureus* DNA in the Assay Control/Negative Control Reagent and the *S. aureus*-specific primers in the CONTROL chamber function as the Internal Control for the assay. During specimen preparation, each patient specimen is added to the Assay Control/Negative Control Reagent and combined with the *S. aureus* DNA prior to amplification. Addition of *S. aureus* DNA to the patient sample allows for parallel processing of target DNA and Control DNA through amplification and detection. The Internal Control monitors amplification inhibition, assay reagent performance and sample processing effectiveness. The Control *S. aureus* target must be amplified and detected in the final reaction or the test is considered invalid and patient results are not reported.

The ***illumi****pro-10™* monitors changes in absorbance characteristics by measuring transmission of light through the Test and Control reaction solutions. Light transmission is checked at the assay Run Start (Signalinitial, Si) and at the assay Run End (Signalfinal, Sf). The ***illumi****pro-10™* calculates the change in light transmission between Run End and Run Start (Sf:Si) and compares the ratio to a fixed cut-off value.

**SPECIMEN:**

**Preferred Sample Types**:

 Nasopharyngeal swabs unpreserved in a sterile tube without medium

Nasopharyngeal swab samples should be collected with suitable swab types (e.g. Polyester, Flocked Nylon or Rayon).

 Place swab(s) in non-nutritive transport medium (eg, Liquid Amies, without charcoal or Liquid Stuart) or store

 unpreserved in a sterile tube without medium.

**Undesirable samples**: Throat swabs, nasal swabs, swabs in medium containing charcoal

**Storage and Transport of Specimens**

Unpreserved samples or samples stored in transport media should be tested as soon as possible, but may be held at room temperature (21–30 C) for up to 5 days or refrigerated (2-8 C) for up to 7 days prior to testing. Do not freeze samples.

**MATERIALS AND EQUIPMENT**:

**MATERIALS PROVIDED IN KIT:**

***illumi****gene* **Pertussis Assay Control/Negative Control Reagent**

***illumi****gene* **Pertussis Test Device**

***illumi****gene* **Pertussis Sample Buffer**

**Mineral Oil**

**ADDITIONAL MATERIALS USED:**

***illumi****gene* Pertussis External Control Kit, Catalog Number: 279930

Disposable latex gloves, powder free

DNase/RNase-free, aerosol resistant pipette tips

Specimen Collection and transport system

**Nasopharyngeal Swabs**

Flocked nylon (Minimum Capacity 69 μL, e.g. Copan 503CS01).

**Non-nutritive Transport Medium** (Maximum Volume: 1.2 mL)

**EQUIPMENT**

Dry-bath with 12 mm heat block capable of 95 C

Digital thermometer with Max/Min Temperature Memory

Vortex mixer

Interval timer

Micropipette capable of dispensing 50 μL

Cutting Equipment

Forceps or tweezers

***iIlumi****pro-10,* Meridian Bioscience

**REAGENT PREPARATION:**

Ensure kit reagents are at room temperature (21 - 30 C) before use. Incorrect results may be obtained if reagents are not brought to room temperature prior to use.

**PRECAUTIONS:**

1. All reagents are for in vitro diagnostic use only.

2. Do not interchange Assay Control/Negative Control Reagent or Test Devices between lots. Sample Buffer and Mineral

 Oil are interchangeable provided they are within assigned expiration dates when used.

3. Follow Biosafety Level 2 and Good Laboratory practices during testing. Treat all specimens and used Test Devices as

 capable of transmitting infectious agents. Do not eat, drink or smoke in areas where specimens or kit reagents are

 handled.

4. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.

5. Quality Control Programs for Molecular Testing Laboratories, including proper use and care of equipment, should be

 employed.

6. Th*e* ***illumi****gene* Pertussis Test Device contains lyophilized reagents. The protective pouch should not be opened until

 ready to perform the assay.

7. The ***illumi****gene* Pertussis Test Device includes a latch feature that is designed to prevent contamination of the test

 area with amplification product. Do NOT use Test Devices with broken latches.

8. Dispose of used ***illumi****gene* Test Devices immediately after processing, leaving the device latch securely in place. Do

 NOT open the Test Device after processing. Opening the device after amplification may result in contamination of the

 test area with amplification product.

**SHELF LIFE AND STORAGE:**

The expiration date is indicated on the kit label. Store the kit at 2 - 30 C.

Do not use the devices or reagents after their expiration dates.

**CALIBRATION:**

There are no calibrations associated with this procedure.

**QUALITY CONTROL:**

1. Each device contains an internal control chamber that controls for amplification inhibition, assay reagents and sample

 processing effectiveness.

2. The heat-treatment step is monitored with an external thermometer and interval timer. Use the max/min

 temperature memory of the thermometer to ensure that a temperature of 95 ± 5 C is maintained. Use the interval

 timer to ensure that heat-treatment duration is 10 ± 2 minutes.

3. Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state

 and local guidelines concerning the running of external quality controls.

4. ***illumi****gene* Pertussis External Control Reagents are supplied separately (Catalog 279930). It is recommended that

 reactivity of each new lot and each new shipment of ***illumi****gene* Pertussis be verified on receipt and before use.

 External control tests should be performed thereafter in accordance with appropriate federal, state and local

 guidelines. The ***illumi****gene* Pertussis test kit should not be used in patient testing if the external controls do not

 produce the correct results.

5. A separate device must be used for each external control reagent.

**QC Testing Frequency and Documentation:**

With each new lot and or shipment

Every 30 days

New lot numbers of external controls must be run against the current Pertusis test kit for cross checking.

**Quality Control Sample Preparation**

**NOTE:** Ensure that the ***illumi****pro-10* is powered on and required performance verifications have been completed prior to initiation of Quality Control Sample Preparation. Refer to the ***illumi****pro-10* Operator’s Manual for further information regarding instrument set-up and operation.

1. Label 1 ***illumi****gene* Pertussis Assay Control/Negative Control Tube for each Control to be tested.

2. **For Negative Control:**

* + Vortex 1 ***illumi****gene* Pertussis Assay Control/Negative Control Tube for a minimum of 10 seconds.

3. **For Positive Control:**

* + Add 50 µL of ***illumi****gene* Pertussis Positive Control to 1 ***illumi****gene* Pertussis Assay Control/Negative Control Tube. Replace and secure the ***illumi****gene* Pertussis Assay Control/Negative Control cap and vortex for a minimum of 10 seconds.

4. Heat each Control Tube in a dry-bath/heat block at 95 ± 5 C for 10 ± 2 minutes. Monitor heat-treatment step with

 digital thermometer and interval timer.

5. Remove each Control Tube from the dry-bath/heat block. Vortex for approximately 10 seconds and proceed to Test

 Procedure.

**Failure of Quality Control Test** The ***illumi****gene* Pertusis test kit should not be used in patient testing if the external controls do not produce the correct results. Results of External QC and action(s) taken when control results are unacceptable are documented on the Illumigene

Log Sheet.

**PROCEDURE:**

**NOTE:** Ensure that the ***illumi****pro-10™* instrument is powered on and required performance verifications have been completed prior to initiation of SPECIMEN PREPARATION. Refer to the ***illumi****pro-10™* Operator’s Manual for further information regarding instrument set-up and operation.

**NOTE:** Laboratory equipment used for handling swabs (eg. scissors, shears, safety snips, forceps, tweezers), should be treated with a molecular grade cleaning agent (eg. 10% bleach), prior to each use. Equipment should be completely dry before handling swabs.

**SPECIMEN PREPARATION:**

1. Place the swab specimen in a labeled Sample Buffer tube. Cut the swab shaft to ensure sample fits in the tube. Elute the sample by vortexing for 45-60 seconds. Remove and discard the swab. Remove the swab and discard immediately in an appropriate waste container, taking care to prevent contamination of samples, equipment and work surfaces.

**Eluted samples may be held at room temperature (21-30 C) for up to 48 hours or refrigerated (2-8 C) for up to 7 days prior to testing.**

1. Add 50 μL of eluted sample to a labeled Assay Control/Negative Control Tube and cap.
2. Repeat Specimen Preparation Steps for all samples to be processed.
3. Vortex each Assay Control/Negative Control Tube containing eluted sample for approximately 10 seconds.
4. Heat each Sample/Control mixture in a dry-bath/heat block at 95 ± 5 C for 10 ± 2 minutes. Monitor heat-treatment step with digital thermometer and interval timer.
5. Remove each Sample/Control tube from the dry-bath/heat block. Heat treated samples may be held at room temperature (21-30 C) for up to 15 minutes prior to testing.
6. Vortex for approximately 10 seconds.

**TEST PROCEDURE:**

**NOTE:** A maximum of 10 samples can be processed in a single ***illumi****pro-10* run.

1. Remove 1 ***illumi****gene* Pertussis Test Device from its protective pouch per sample. Carefully open the device, holding the chambers such that the lyophilized reagent will not fall out upon opening. Place device on a flat surface or in a rack that can accommodate the device.
2. Transfer 50 μL of the heat-treated sample to the TEST chamber (White Bead) of the ***illumi****gene* Test Device. Take care not to introduce air to the reaction mixture. Using a new pipette tip, transfer 50 μL of the heat-treated sample to the CONTROL chamber (Yellow Bead) of the ***illumi****gene* Test Device. Take care not to introduce air to the reaction mixture.
3. Add 1 drop of Mineral Oil to both the TEST chamber and CONTROL chamber. Close the ***illumi****gene* Test Device and fasten the latch securely.
4. Tap device on the bench top or mix to remove air bubbles. Carefully examine the Test Device for rehydration of the Control/Test Bead, for air bubbles left in the chamber and liquid in the top of the device. If undissolved beads, air bubbles or liquid in the top of the device are noted, tap the device on the bench top and repeat visual inspection. Amplification and detection should be initiated within 15 minutes.
5. Insert the ***illumi****gene* Test Device into the ***illumi****pro-10* and initiate amplification reaction and detection. Results will be displayed at the conclusion of the run.

**INTERPRETATION OF RESULTS:**

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| --- | --- | --- |
| **Sample ID**  | **Reported Result**  | **Interpretation**  |
| Patient Specimen  | POSITIVE  | Sample contains *Bordetella pertussis* IS481 target DNA.a |
| NEGATIVE | No Bordetella pertussis DNA detected.  |
| INVALID | **No reportable result. Repeat the test using the original sample.** Inhibitory patient specimen, improper sample preparation, reagent failure, instrument failure or internal control failure. If second test of original sample gives an invalid result , notify car giver or laboratory to recollect specimen if indicated. |
| Positive Control | POSITIVE | Valid positive control result. Reagents active at time of use, ***illumi****pro-10* performing correctly.  |
| NEGATIVE | **Incorrect control result.** Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian’s Technical Services Department at 1-800-343-3858 (US) or your local distributor.  |
| INVALID | **No reportable result. Repeat entire assay run using original samples.** Improper sample preparation, reagent failure, instrument failure or internal control failure.  |
| Negative Control | POSITIVE | **Incorrect control result.** Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian’s Technical Services Department at 1-800-343-3858 (US) or your local distributor.  |
| NEGATIVE | Valid negative control result. Reagents active at time of use, ***illumi****pro-10* performing correctly.  |
| INVALID | **No reportable result. Repeat entire assay run using original samples.** Improper sample preparation, reagent failure, instrument failure or internal control failure.  |
| EMPTY WELL | NONE  | No ***illumi****gene* Test Device in the ***illumi****pro-10* Well. **OR** The ***illumi****gene* Test Device present is compromised due to sample preparation failure, dirty device or improperly seated device. **Repeat the test using original sample.**  |

aIS481 is found in multiple copies in *B. pertussis* (50 to 238 copies/genome), in *B. holmesii* (8 to 10 copies/genome) and less frequently in *B. bronchiseptica.*

**CALCULATIONS:**

There are no calculations associated with this procedure.

**REPORTING OF RESULTS:**

**Positive Test:** Sample contains *Bordetella pertussis* target DNA

**Negative Test:** No *Bordetella pertussis* DNA detected

**EXPECTED RESULTS:**

Overall incidence of *B. pertussis* as detected by the ***illumi****gene* Pertussis Assay in prospectively and retrospectively collected, non-selected specimens (all comers) during the period of this study was 8.2% (57/692).

**LIMITATIONS OF THE PROCEDURE:**

1. 1. The ***illumi****gene* Pertussis assay targets the IS481 insertional element of the Bordetella genome. The IS481 insertional element is present in *B. pertussis, B. holmesii* and some strains of *B. bronchiseptica*.
2. This product can be used only with the ***illumi****pro-10* instrument.
3. The ***illumi****gene* Pertussis DNA assay is a qualitative assay and does not provide quantitative values or information about organism load.
4. This device has not been evaluated for monitoring treatment of *Bordetella pertussis* infections.
5. This test has not been evaluated for specimens other than nasopharyngeal swab specimens, for immunocompromised individuals or from patients not suspected of infection with *Bordetella pertussis*.
6. Results from this test must be correlated with the clinical history, epidemiological data, and any other data available to the clinician.
7. Prevalence of *Bordetella pertussis* will affect the positive and negative predictive values for the assay.
8. *Bordetella parapertussis* which causes a pertussis-like illness is not detected by the ***illumi****gene* Pertussis DNA assay. Illness caused by *B. parapertussis* is generally milder than illness caused by *B. pertussis* because the bacteria do not produce pertussis toxin.
9. Respiratory infections can be caused by *Bordetella pertussis* as well as other pathogens. Positive results do not preclude coinfection with other respiratory pathogens. False-negative *Bordetella pertussis* results are more likely if patients are tested later in the disease course (more than two weeks after symptom onset), due to declining Bordetella DNA. False-negative results may also be increased in patients treated with antibiotic therapy.
10. Environmental contamination of an exam room from a prior patient or a recent pertussis vaccination administration may result in false-positive test results.
11. The detection of nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedure in any one of these steps can lead to incorrect results.
12. Organism nucleic acids may persist *in vivo*, independent of organism viability. The ***illumi****gene* Pertussis assay does not distinguish between viable and nonviable organisms.
13. As with all molecular based diagnostic tests, (A) False negative results may occur from the presence of inhibitors, technical error, sample mix-up or low numbers of organisms in the clinical specimen; (B) False positive results may occur from the presence of cross-contamination by target organisms, their nucleic acids or amplified product, and from non-specific signals.
14. Acetyl salicylic acid, as found in aspirin, produced invalid results when tested at concentrations above 5 mg/mL during *B. pertussis* strain BAA-589 Limit of Detection replicate testing.

**PERFORMANCE CHARECTERISTICS:**

Refer to Directional Insert- Meridian Bioscience ***illumi****gene Pertussis*

**REFERENCES:**

Refer to Directional Insert- Meridian Bioscience ***illumi****gene Pertussis*

**For technical assistance**, call Technical Support Services at 800- 343-3858 between the hours of 8AM and 6PM, USA Eastern Standard Time.

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| **Date:** | **Author:** | **Date:** | **Validated by:** | **Date:** | **Approved by:** |
| 05/21/2014 | Elaine Krueger |  |  |  |
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| **Annual Review** |
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