CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Bordetella Pertussis
- Method change –

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Ordering Information:

Mnemonics:	**B. Pertussis Gp**
Test Name:	B. Pertussis Group
Test Number:	7000900
Specimen:	Nasopharyngeal swab specimen collection should be performed in accordance with institutional procedures for collection of clinical specimens for *Bordetella pertussis* infection. Nasopharyngeal swab samples should be collected in e-Swab transport medium.

Effective Date: Testing will begin on Monday, August 17, 2015 with samples received after testing is completed on Saturday, August 15, 2015.

Performed: Monday through Saturday

Reference Range: Not Detected

Background:

Clinical Significance:
Pertussis, commonly known as whooping cough, is a highly contagious respiratory illness caused by *Bordetella pertussis*. It is usually spread by coming in close contact to aerosolized droplets from an infected person who coughs or sneezes. The disease and its symptoms can last for several weeks to months, but symptoms may not always be present, making pertussis clinically indistinguishable from other respiratory illnesses. The severe complications of pertussis make a rapid, accurate diagnosis even more important.

Principle of Test:
The ***illumi****gene* Pertussis DNA Amplification Assay is based on loop-mediated amplification (LAMP) technology. The assay targets a 198 base pair (bp) sequence of the *Bordetella pertussis* genome residing in a region of the IS481 insertional element sequence. This test has been FDA approved as an in-vitro diagnostic test.

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. Reaction solution absorbance characteristics are monitored by the Meridian ***illumi****pro-10*™ Incubator/Reader. 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.

Results Interpretation:

Respiratory infections can be caused by Bordetella pertussis as well as other pathogens. Positive results do not preclude coinfection with other respiratory pathogens. Negative results for the ***illumi***gene Pertussis DNA Amplification Assay do not preclude *Bordetella pertussis* infection. 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.

Bordetella parapertussis, which causes a pertussis-like illness, is not detected by the illumigene 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. CPAL will store the original samples for at least 7 days following testing. If Bordetella parapertussis testing is desired, please contact the submitting institution within 7 days of collection to have the testing added.

Limitations:

1. The illumigene 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. The illumigene Pertussis DNA assay is a qualitative assay and does not provide quantitative values or information about organism load.
2. This device has not been evaluated for monitoring treatment of Bordetella pertussis infections.
3. 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.
4. Results from the ***illumi****gene* Pertussis assay should be used in conjunction with information obtained during the patient's clinical evaluation as an aid in diagnosis of *Bordetella pertussis* infection and should not be used as the sole basis for treatment or other patient management decisions.
5. Environmental contamination of an exam room from a prior patient or a recent pertussis vaccination administration may result in false-positive test results.
6. 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.
7. Organism nucleic acids may persist in vivo, independent of organism viability. The illumigene Pertussis assay does not distinguish between viable and nonviable organisms.
8. 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.
9. 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.

Validation Data:

Accuracy and Reproducibility:
Within-run and between-run reproducibility were performed over five days. Within-run reproducibility was verified by testing a known positive and a known negative specimen in duplicate in the same run. Between-run reproducibility was verified by repeating the known positive and known negative for four additional days by a minimum of two different operators. All results reproduced as expected.

In additional, positive and negative controls were run for 20 days to validate the test's internal control. All results reproduced as expected.

Method Comparison:
Method comparison studies included testing a panel of twenty specimens, including ten positive and ten negative specimens provided by Meridian. Results obtained for all samples were as expected.

In addition, 17 patient samples collected in E-swab media were aliquotted for CPAL method comparison studies prior to sending to Quest for analysis. The result of one sample was not in agreement. Upon duplicate repeat testing, the sample tested positive for 1 of 3 replicates. The specimen was sent to Meridian for resolution and was determined to have CFU near the cutoff value. One other sample was QNS for resolution of an initially "invalid" result. All others were in agreement with the Quest result.

Limit of Detection:
Limit of Detection (LOD) studies were performed at CPAL to verify the manufacturer's claim of 3265 CFU/mL (1.48 CFU/test). Two swabs with known concentrations of *Bordetella pertussis* were sent from Meridian to perform the testing. Dilutions were made using E-Swab liquid Amies media. Duplicate testing of individual swabs/dilutions at three levels near the LOD was performed and the manufacturer's claim was verified.

Suggested Coding:

Suggested CPT Code Update	**87798 (x1)**
LOINC Code Update	**43913-3** (B. pertussis DNA in nasopharynx)
CAP Test Activity Code	5042 (B. pertussis, NAA, FDA approved)

References:
illumi*gene Pertussis* Package Insert. Meridian Bioscience.
Validation Documentation for: ***illumi****gene*® Pertussis on the Meridian ***illumi****pro*-10. CPAL 2015