



***Bordetella pertussis* and *Bordetella parapertussis* Detection by PCR Now Available at PAML**

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The Organisms

Bordetella pertussis and *Bordetella parapertussis* are gram-negative coccobacilli with a propensity for localization in ciliated epithelial cells of the respiratory tract. While both are exclusively human respiratory tract pathogens transmissible by aerosolized respiratory secretions, *B. pertussis* causes a highly communicable infection in individuals of all ages, commonly referred to as pertussis or whooping cough. Conversely, *B. parapertussis* infection is generally associated with a milder, less prevalent form of the disease.

The Disease

Classic pertussis in unimmunized individuals is manifested in three stages: the catarrhal, paroxysmal, and convalescent stages. The paroxysmal stage is characterized by severe, repetitive coughing and culminates in the hallmark inspiratory whoop. However, most infants or young children who have been partially immunized or older children and adults with waning immunity do not experience classical signs and symptoms. Rather, these individuals may be asymptomatic or present with nonspecific symptoms that mimic the common cold, thus complicating the diagnosis of the disease.

The Incidence

Pertussis is endemic in the United States, largely due to the waning immunity from vaccination or previous disease. *B. pertussis* has an attack rate greater than 90% in unimmunized populations. Pertussis was a major cause of infant and childhood morbidity and mortality in the prevaccine (DTP) era. Today, severe morbidity is most commonly seen in young infants, and the majority of fatalities occur in unimmunized children less than 1 year of age. The disease appears to be perpetuated by asymptotically infected young adults or adults experiencing mild respiratory symptoms.

Diagnostic Testing: Culture/DFA/PCR

Although culture of *B. pertussis* by conventional methods can be highly sensitive and specific, it is inherently challenging. Culture is most sensitive in the acute stage of the disease; however, many individuals do not seek medical intervention while acutely ill. Other important factors that reduce culture sensitivity include recent antibiotic therapy, poor specimen quality, inappropriate specimen transport conditions, and some degree of immunity. In addition, culture is a slow process, requiring 3-6 days to detect *B. pertussis* isolates and 2-4 days to detect *B. parapertussis* isolates. Also, the solid medium used for specimen transport and culture has a relatively short shelf life. Direct fluorescent antibody (DFA) testing provides a rapid alternative to culture; however, DFA sensitivity compared to culture reportedly varies from 40%-75%, depending on the lab's proficiency, and has a specificity of approximately 90%. Polymerase chain reaction (PCR) detection offers a sensitivity and specificity that transcends that achievable by culture or DFA and has quickly replaced culture as the diagnostic "gold standard."

Quick Facts

- ▶ PCR is the "gold standard" for *B. pertussis* detection.
- ▶ LightCycler™ technology provides rapid, sensitive, and specific detection of *Bordetella* DNA.
- ▶ Assay sensitivity is 1 organism per 5 µL of processed specimen.
- ▶ Dacron or rayon swabs are required for specimen collection. *Calcium alginate swabs are unacceptable.*

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LightCycler™ Technology

Recent advancements in PCR detection methods have facilitated the development of rapid *B. pertussis* and *B. parapertussis* detection assays. LightCycler™ technology is predicated on real-time detection of PCR products by fluorescence resonance energy transfer (FRET) technology. The process entails the use of two probes each labeled with a different fluorophore. When the probes come into proximity of each other (i.e., when each binds to its complementary target), a fluorescence signal is emitted and measured by the instrument. The process occurs during each cycle of PCR, allowing for continuous monitoring of amplification.

Test Design

Detection of *B. pertussis* and *B. parapertussis* occurs simultaneously in a single reaction tube using the Bordetella-specific insertion sequences, IS481 and IS1001, as targets. The IS481 sequence occurs in *B. pertussis* at a frequency of 50-100 copies per organism, and the IS1001 sequence occurs at a frequency of 20-50 copies per *B. parapertussis* organism. The assay has an analytic sensitivity of approximately 1 organism of *B. pertussis* (approximately 5 copies of the IS481) or *B. parapertussis* (approximately 10 copies of IS1001) per 5 microliters of processed specimen. A negative result does not rule out the possibility of the presence of PCR inhibitors in the patient specimen nor the presence of *B. pertussis* or *B. parapertussis* DNA below the limit of detection of the assay.

Test Information

DESCRIPTION	BORDETELLA PERTUSSIS/PARAPERTUSSIS BY PCR
METHOD	PCR
ORDER CODE	BORPCR
CPT CODE	87801
SPECIMEN	Collect 1 NP swab (Dacron or rayon tip with wire shaft) by inserting the swab through the nose into the posterior nasopharynx and rotating for at least 5 seconds. Or collect a nasopharyngeal wash. Place swab or wash in sterile, capped tube. Store and transport refrigerated.
COMMENTS	<i>Unacceptable conditions:</i> Swabs that are frozen, at room temperature, or in transport media. Calcium alginate is a known inhibitor of PCR and thus not acceptable. <i>Stability:</i> 7 days refrigerated.
SCHEDULE	Monday – Friday
TURNAROUND	2-5 days
REPORT	Result: Negative for <i>Bordetella pertussis</i> DNA. Negative for <i>Bordetella parapertussis</i> DNA. Comment Method Comment

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