Bordetella pertussis and Bordetella parapertussis Detection by Real-Time PCR

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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 one year of age. The disease appears to be perpetuated by symptomatically 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."

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Quick Facts
- PCR is the “gold standard” for B. pertussis detection.
- Real-Time PCR technology provides rapid, sensitive, and specific detection of Bordetella DNA.
- Assay sensitivity is 1 organism per 3 μL of processed specimen.
- Dacron or rayon swabs are required for specimen collection. Calcium alginate swabs are unacceptable.
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Real-Time PCR Technology
Recent advancements in PCR detection methods have facilitated the development of rapid B. pertussis and B. parapertussis detection assays. Real-Time PCR technology allows for the simultaneous amplification and detection of target sequences. Amplified target sequences are detected by target-specific probes after each cycle of PCR.

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 per 3 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.

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