



Laboratory Diagnosis of *Bordetella* Infections: Comprehensive Methods for Species Identification and Clinical Significance

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DOI: 10.31080/ASMI.2026.09.1635

Received: August 12, 2026

Published: October 05, 2026

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Abstract

Bordetella species are important respiratory pathogens requiring accurate laboratory identification for appropriate clinical management. This comprehensive review examines current diagnostic methods including culture, molecular techniques, and serology for the detection and differentiation of pathogenic *Bordetella* species. With the evolution of molecular diagnostics, laboratories can now reliably distinguish between *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and emerging species such as *B. holmesii*. Each species presents unique clinical challenges and antimicrobial susceptibility patterns, making accurate identification essential for optimal patient care and public health surveillance.

Keywords: *Bordetella pertussis*; Whooping Cough; Pertussis Diagnosis; Polymerase Chain Reaction; Nasopharyngeal Culture; Serological Methods

Introduction

The genus *Bordetella* comprises gram-negative coccobacilli that primarily affect the respiratory tract of humans and animals. Currently, nine species are recognized within the genus, with four species (*B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. holmesii*) being the most clinically significant for human infections.

Accurate species identification is crucial for several reasons: different species exhibit varying antimicrobial susceptibility patterns, have distinct epidemiological characteristics, and may require different treatment approaches. *B. pertussis* remains the most important cause of pertussis (whooping cough), while *B. parapertussis* causes a milder but similar illness. *B. bronchiseptica*

primarily affects animals but can cause severe infections in immunocompromised humans, and *B. holmesii* has emerged as an occasional cause of pertussis-like illness.

Laboratory diagnosis has evolved significantly over the past decades, with molecular methods now playing a central role alongside traditional culture and serological techniques. This review examines current diagnostic approaches, their advantages and limitations, and the clinical significance of accurate species identification. The methods used for this review included a search of PubMed, MEDLINE, and relevant reference lists using the terms '*Bordetella* diagnosis', 'pertussis laboratory methods', '*Bordetella* PCR', and 'pertussis serology', restricted to English-language publications.

Overview of clinically relevant *Bordetella* species

Bordetella pertussis

B. pertussis is the primary causative agent of pertussis (whooping cough), a highly contagious respiratory infection characterized by paroxysmal cough, post-tussive vomiting, and inspiratory whooping in severe cases. The organism is strictly human-adapted and produces pertussis toxin (PT), which is unique to this species and serves as both a virulence factor and a diagnostic marker [1]. It is non-motile, oxidase-positive, and requires special culture media for primary isolation, typically growing within 3–7 days. It is susceptible to macrolides and is the target of current acellular pertussis vaccines.

Bordetella parapertussis

B. parapertussis causes a pertussis-like syndrome that is generally milder than classical pertussis. The human-adapted strain (*B. parapertussis hu*) is distinguished from the ovine strain (*B. parapertussis ov*) that affects sheep. Unlike *B. pertussis*, it does not produce pertussis toxin, which has important diagnostic and clinical implications [2]. It grows more rapidly and can be differentiated by urease production and growth on simple media.

Bordetella bronchiseptica

B. bronchiseptica primarily affects animals but can cause severe infections in immunocompromised humans, particularly those with animal contact. Unlike the other two species above, it is motile, grows rapidly on simple media, and is catalase-positive. It exhibits different antimicrobial susceptibility patterns, often demonstrating resistance to macrolides [3].

Bordetella holmesii

B. holmesii is a more recently recognized pathogen that can cause pertussis-like illness, particularly in adolescents and young adults, and has been associated with bacteremia in asplenic patients [4]. It shares genetic similarities with other *Bordetella* species but has distinct diagnostic markers allowing species identification.

Culture methods

Culture media and growth requirements

Culture remains the gold standard for *Bordetella* diagnosis, providing 100% specificity and enabling antimicrobial susceptibility testing. However, sensitivity varies significantly based on timing of specimen collection, with optimal results obtained within the first two weeks of illness [5].

Regan-Lowe medium (charcoal horse blood agar with cephalexin 40 µg/ml) is currently the most widely used medium for isolation, as charcoal neutralizes toxic metabolites while cephalexin suppresses normal respiratory flora. CLAT medium (charcoal horse blood agar with cephalexin, amphotericin B, and trimethoprim) offers enhanced selectivity. The traditional Bordet-Gengou medium, though historically important, has largely been replaced due to contamination issues and lower sensitivity.

Culture identification and species differentiation

B. pertussis colonies are typically small (1–3 mm), smooth, convex, and have a pearl-like appearance with a characteristic hemolysis pattern, requiring 3–7 days and enhanced humidity/CO₂. Key biochemical characteristics are summarized in Table 1.

Characteristic	<i>B. pertussis</i>	<i>B. parapertussis</i>	<i>B. bronchiseptica</i>	<i>B. holmesii</i>	Growth rate
Oxidase	Positive	Positive	Positive	Positive	3–7 days
Urease	Negative	Positive	Positive	Negative	2–4 days
Nitrate reduction	Negative	Negative	Positive	Positive	1–2 days
Motility	Non-motile	Non-motile	Motile	Non-motile	3–5 days
Pertussis toxin production	Positive	Negative	Negative	Negative	Variable

Table 1: Biochemical characteristics and growth rates of clinically relevant *Bordetella* species.

Abbreviation: CO₂, carbon dioxide.

Molecular diagnostic methods

Molecular diagnostics have revolutionized *Bordetella* detection, offering superior sensitivity and specificity compared to culture methods. Real-time PCR has become the method of choice for rapid diagnosis, particularly during the early stages of illness [6].

Target sequences for molecular detection

- **IS481 (insertion sequence 481):** The most widely used target for *B. pertussis* detection, present in 50–238 copies per genome, providing excellent analytical sensitivity. However, IS481 is also present in approximately 1–5% of *B. bronchiseptica* strains and in *B. holmesii* (8–10 copies), which can lead to cross-reactivity and requires confirmatory testing [7].
- **IS1001:** Specific for *B. parapertussis*, present in 35–50 copies per genome, providing excellent discrimination for species identification.
- **Pertussis toxin promoter (ptxS1):** Highly specific for *B. pertussis* as pertussis toxin production is unique to this species. Often used in combination with IS481 to enhance specificity.

- **Filamentous hemagglutinin gene (FHA):** Present in all *Bordetella* species but with sequence variations allowing for species differentiation through melting curve analysis.
- **hIS1001:** An insertion sequence similar to IS1001 but specific for *B. holmesii*, enabling identification of this emerging pathogen.

Multiplex PCR approaches

Modern multiplex PCR assays can simultaneously detect and differentiate multiple *Bordetella* species in a single reaction. Standard multiplex panels include IS481, IS1001, and ptxS1 to detect *B. pertussis* and *B. parapertussis*. Extended panels add hIS1001 and additional targets for *B. holmesii* and *B. bronchiseptica*, providing comprehensive coverage. Current multiplex assays achieve analytical sensitivities of 1.0–2.7 genome equivalents per microliter, with clinical sensitivities of 85–95% depending on timing of specimen collection [8].

Species differentiation algorithms

Accurate species identification requires interpretation algorithms based on the combination of target sequences detected, as summarized in Table 2.

Species	IS481	IS1001	ptxS1	hIS1001	Interpretation
<i>B. pertussis</i>	+	–	+	–	Classic pertussis
<i>B. parapertussis</i>	–	+	–	–	Parapertussis
<i>B. holmesii</i>	+	–	–	+	Holmesii infection
<i>B. bronchiseptica</i>	+/–	–	+	–	Bronchiseptica

Table 2: Multiplex PCR species differentiation algorithm.

Note: +/- indicates that some *B. bronchiseptica* strains (1–5%) may carry IS481, requiring additional targets for definitive identification. +, positive; –, negative; PCR, polymerase chain reaction.

Serological methods

Serological testing plays a crucial role in *Bordetella* diagnosis, particularly for late-stage infections (>2–4 weeks) when culture and PCR sensitivity may be reduced. Serology is based on detection of specific antibodies against *Bordetella* antigens, with pertussis toxin being the most important target due to its specificity for *B. pertussis* [9].

Pertussis toxin antibodies

IgG anti-PT is the most commonly used serological marker. Single serum samples with IgG anti-PT levels ≥100 IU/mL suggest recent infection or vaccination. Paired sera showing a ≥4-fold rise provide stronger evidence of acute infection. IgA anti-PT, with cut-off values of ≥15–20 IU/mL, may be useful for diagnosing recent infection, as IgA responses are shorter-lived. Recent vaccination (within 1–3 years) can interfere with interpretation, as vaccine-induced antibodies cannot be distinguished from infection-induced antibodies.

Filamentous hemagglutinin antibodies

FHA is present in all pathogenic *Bordetella* species, making anti-FHA antibodies less specific than anti-PT antibodies. However, FHA serology can be useful for detecting *B. parapertussis* infections, which do not produce pertussis toxin. Increases in anti-FHA

antibodies without corresponding anti-PT increases may suggest *B. parapertussis* infection.

Interpretation guidelines

Anti-PT IgG level	Interpretation	Clinical action
<40 IU/mL	Negative	No evidence of recent infection
40–100 IU/mL	Borderline	Consider clinical context
≥100 IU/mL	Positive	Recent infection or vaccination
≥4-fold rise	Significant increase	Strong evidence of infection

Table 3: Interpretation of anti-PT IgG serology results.

Abbreviations: IgG, immunoglobulin G; IU, international units; PT, pertussis toxin.

Specimen collection and processing

Optimal specimen types

Nasopharyngeal specimens are the gold standard for *Bordetella* detection, as organisms primarily colonize the posterior nasopharynx. Synthetic fiber swabs with flexible aluminum or plastic shafts should be used (cotton swabs are inhibitory). The swab should be inserted through the nostril to the posterior nasopharynx, rotated gently, and held for 10–15 seconds before slow removal. Anterior nasal swabs are less optimal but may be acceptable for molecular testing. Throat swabs are not recommended due to significantly lower sensitivity [5].

Transport and storage

- **For culture:** Specialized transport media (Regan-Lowe transport medium or charcoal-containing transport media) should be used, with processing within 48 hours at 4°C. Freezing of specimens intended for culture should be avoided.
- **For molecular testing:** Universal transport medium (UTM) or viral transport medium (VTM) is acceptable, with storage at 4°C for up to 48 hours or frozen at –20°C for longer periods.
- **For serology:** Serum specimens should be collected in serum separator tubes, processed promptly, and stored at 4°C for short-term or –20°C for extended storage. Paired sera should be collected 2–4 weeks apart.

Diagnostic algorithm and testing strategy

The choice of diagnostic method depends on several factors including duration of symptoms, patient age, vaccination status,

and clinical presentation. An integrated approach using multiple methods often provides the most accurate diagnosis.

First-line testing

- **Early disease (0–2 weeks):** Real-time PCR is the method of choice, offering the highest sensitivity during early illness. Culture should be performed concurrently, especially during outbreak investigations, to enable antimicrobial susceptibility testing and molecular epidemiology.
- **Late disease (>2–4 weeks):** Serology becomes the primary diagnostic method as bacterial loads decrease and antibody responses develop. PCR may still be attempted but sensitivity is significantly reduced.

Special populations

- **Infants <6 months:** PCR and culture are preferred as maternal antibodies may interfere with serological interpretation. These patients are at highest risk for severe disease and require prompt diagnosis.
- **Recently vaccinated individuals:** Avoid serological testing within 1–3 years of vaccination. Rely on PCR and culture for accurate diagnosis.
- **Immunocompromised patients:** Consider extended PCR panels including *B. bronchiseptica* and *B. holmesii*. These patients may have prolonged bacterial shedding, making culture and PCR useful for extended periods.

- **Contact investigation:** Serology may be useful for identifying asymptomatic or mildly symptomatic contacts during outbreak investigations, particularly in adults with atypical presentations.

Clinical significance and treatment implications

Bordetella pertussis

B. pertussis causes the most severe form of pertussis, with highest morbidity and mortality in infants <6 months of age. The illness is characterized by three stages: catarrhal (1–2 weeks), paroxysmal (2–6 weeks), and convalescent (weeks to months). First-line therapy includes macrolides (erythromycin, azithromycin, clarithromycin). Treatment is most effective when started during the catarrhal stage but may reduce transmission even when started later. Alternative agents include trimethoprim-sulfamethoxazole [1]. Cases must be reported to public health authorities.

Bordetella parapertussis

B. parapertussis generally causes milder illness with shorter duration. Macrolides remain first-line therapy, though treatment may be less critical given the generally milder course. Notably, current pertussis vaccines do not protect against *B. parapertussis* infection, which may contribute to its persistence in vaccinated populations [2].

Bordetella bronchiseptica

B. bronchiseptica often shows macrolide resistance, requiring tetracyclines (doxycycline) or fluoroquinolones (ciprofloxacin) as alternatives. Trimethoprim-sulfamethoxazole may also be used. Investigation of animal contacts is important, as infected pets may require veterinary treatment to prevent continued human exposure [3].

Bordetella holmesii

Limited clinical data exist, but *B. holmesii* appears susceptible to macrolides and fluoroquinolones. Treatment recommendations are extrapolated from other *Bordetella* species and *in vitro* susceptibility data [4].

Quality assurance and laboratory considerations

Analytical performance requirements

Laboratories performing *Bordetella* testing should establish analytical performance characteristics including analytical sensitivity, specificity, precision, and accuracy. Participation in external quality assessment programs is essential [10].

For PCR quality control, appropriate positive and negative controls should be included with each run, with monitoring for PCR inhibition using internal controls. For culture quality control, reference strains should be used for media quality control. For serology, calibrated assays with appropriate reference standards should be used, ensuring proper sample handling to prevent antibody degradation.

Contamination prevention

Due to the high sensitivity of molecular methods, strict contamination control measures must be implemented:

- Physical separation of pre- and post-PCR areas
- Separate equipment and reagents for each area
- Unidirectional workflow from sample preparation to result reporting
- Regular decontamination of work surfaces and equipment
- Appropriate negative controls to detect contamination.

Future directions and emerging technologies

Next-generation sequencing

Whole genome sequencing (WGS) and targeted metagenomic approaches offer new opportunities for comprehensive pathogen detection and characterization, providing detailed molecular epidemiological information and detecting antimicrobial resistance genes directly from clinical specimens. Culture-independent sequencing methods are being developed that can provide strain-level identification without bacterial isolation, potentially revolutionizing outbreak investigation and surveillance.

Point-of-care testing

Development of rapid point-of-care molecular tests could enable immediate diagnosis and treatment decisions, particularly important for pediatric patients who may benefit from early

antimicrobial therapy. These tests must maintain the sensitivity and specificity of laboratory-based methods while providing results within hours.

Multiplex respiratory panels

Integration of *Bordetella* detection into comprehensive respiratory pathogen panels allows simultaneous testing for multiple causes of respiratory illness. These panels must include appropriate *Bordetella* species targets to ensure comprehensive coverage and accurate diagnosis.

Conclusion

Laboratory diagnosis of *Bordetella* infections has evolved significantly with the development of sensitive and specific molecular methods. While culture remains essential for antimicrobial susceptibility testing and outbreak investigations, molecular diagnostics now serve as the primary method for rapid detection and species identification. Accurate species identification is critical for appropriate clinical management, as different *Bordetella* species exhibit distinct antimicrobial susceptibility patterns and clinical presentations. The integration of molecular methods, culture, and serology provides comprehensive diagnostic capabilities addressing the needs of different clinical scenarios and patient populations. Effective *Bordetella* diagnosis requires close collaboration between clinical laboratories, healthcare providers, and public health authorities to ensure appropriate test utilization, accurate result interpretation, and effective disease control measures.

Acknowledgments

None.

Conflict of Interest

None declared.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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