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Detection of *Bordetella holmesii* Using *Bordetella pertussis* IS*481* PCR Assay

After our manuscript comparing the performance of PCR, culture, and direct fluorescent-antibody testing for *Bordetella pertussis* (3) went to press, we obtained six isolates of *Bordetella holmesii* for the purpose of testing with our *B. pertussis* IS*481* PCR assay. This request for isolates was prompted by the recent report by Yih et al. (6) on the isolation of *B. holmesii* from nasopharyngeal (NP) specimens from patients with pertussis-like cough. Previously, *B. holmesii* has been associated with septicemia in immunocompromised patients (5) and was isolated from the sputum of patients with respiratory failure (4). Yih et al. isolated *B. holmesii* infrequently from NP specimens during their evaluation (0.26% positivity rate, compared to 6.6% positivity rate for *B. pertussis*) (6).

B. holmesii isolates (kindly provided by H. George, Massachusetts Department of Public Health) were grown on Trypticase soy agar plates containing 5% sheep blood for 48 h at 35°C. Colony picks were suspended in sterile water and were tested with the *B. pertussis* PCR assay as previously described (3). Initially, the quantity of organisms added to PCRs was not determined, but it was estimated to be at least 100 to 1,000 CFU per reaction. All six *B. holmesii* isolates generated strong positive results with the *B. pertussis* IS*481* PCR assay. To evaluate the limit of detection, suspensions of two *B. holmesii* isolates were prepared, and cell concentrations were estimated based on McFarland turbidimetric standards. Aliquots of serial dilutions were tested with the *B. pertussis* PCR assay. The analytical sensitivity ranged from 0.06 to 0.3 CFU per PCR, which is similar to the sensitivity we previously reported for *B. pertussis* (3).

B. pertussis PCR assays that target the IS*481* repetitive element may cross-react with *B. holmesii*. Our PCR assay utilizes previously described primers and probe. The sequences of the upstream and downstream primers were previously described by Glare et al. (1) and He et al. (2), respectively. The sequence of our capture probe is identical to that of the downstream primer used by Glare et al. (1). We have not determined whether actual NP specimens from patients infected or colonized with *B. holmesii* would generate a positive result with our *B. pertussis* PCR test. We have not isolated *B. holmesii* from NP swab specimens in our laboratory. However, our Regan-Lowe transport medium contains 40 μg of cephalexin per ml, to which *B. holmesii* is reportedly susceptible (E. Mazengia, H. George, E. Silva, and J. Peppe, Abstr. 99th Gen. Meet. Am. Soc. Microbiol. 1999, abstr. A-113, p. 24, 1999). It is possible that some of the patients in our earlier study (3) who were positive by PCR alone were infected or colonized with *B. holmesii* and not with *B. pertussis*. However, if the *B. holmesii* prevalence in our study were similar to that reported in Massachusetts, the number of positive specimens would be very low.

The clinical and public health significance of *B. holmesii* as a respiratory pathogen in otherwise healthy individuals remains to be determined. In fact, it has yet to be proven conclusively that *B. holmesii* causes respiratory disease. Nonetheless, these data suggest that *B. holmesii* may confound *B. pertussis* PCR assays that target IS*481*, requiring redefinition of performance characteristics or modification of assay methods.

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Mike J. Loeffelholz*
Curt J. Thompson
Karla S. Long
Mary J. R. Gilchrist
State Hygienic Laboratory
University of Iowa
Iowa City, Iowa 52242

*Phone: (319) 335-4500
Fax: (319) 335-4555
E-mail: michael-loeffelholz@uiowa.edu